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May 1, 1985

Final Report for
Cooperative Agreement No. 58-7B30-0216

Environmental Toxicants in Grain Dusts

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Abstract

Grain dust from grain elevator facilities was sampled and analyzed in order to determine its potential determental effects on those exposed to it. A total of 174 respirable (dust $\leq$ 10 μm in aerodynamic diameter) and 60 settled grain dust samples were collected. Respirable and settled dust samples contained fungi similar in number, but different in species to previous studies. Bacteria were few in respirable dust, but numerous in settled dust. Endotoxins, a toxic component of Gram-negative bacterial cell walls, were detected in higher concentrations that had been previously reported, but still not in concentrations high enough to elicit a febrile or respiratory response under the conditions that we sampled. Malathion, a pesticide, was also detected in settled grain dust, but not diazinon. As with endotoxins, the concentrations of malathion detected were not high enough to exceed threshhold limit values for occupational exposures nor comparable guidelines for animal feed.

Analyses for mycotoxins, toxic metabolites of fungi, revealed that over 90% of the samples contained one of the following three mycotoxins: aflatoxin B_1, zearalenone, or secalonic acid D. Secalonic acid D was frequently detected in grain dust despite the lack of reports of it in grain. The concentrations of the mycotoxins were relatively low. The occurrence of a particular toxin varied from year to year. One mycotoxin, secalonic acid D, was chosen for animal studies and enhanced the mortality rate of mice from influenza virus more than just additively. Secalonic acid D appeared to affect the immune functions of mice as evidenced by reduced antibody titers to sheep red blood cells and to influenza virus and by reduced mitogen - stimulation of lymphocyte blastogenesis.

I. INTRODUCTION

Grain shipping is of great economic importance in southern Louisiana and since the recent slump in oil shipment, grain has been the major item exported from these ports. In 1982, 34.6 million short tons of grain and grain products were exported from terminal grain elevators located along the Mississippi River near New Orleans. Some of these elevators are more than 20 years old, while others are less than five years old. Their dust control systems vary widely in efficiency with some, even in older elevators, being very efficient. Within each elevator, some areas are dustier than others, e.g., where grain is unloaded from barges or trains, cleaned and weighed, and loaded onto ships. No work area was totally without dust, although many had relatively low levels.

Airborne and settled dusts in grain elevators and their associated storage/transfer facilities in the lower Mississippi River area are heterogenous substances containing particles abraded from cereal grains, such as corn and wheat, and from soybeans. Large numbers of fungal spores are present, as well as bacteria, pollen grains, various living mites, insects or their body parts, mammalian debris, and various chemical additives such as pesticides and herbicides (Kozlowski, 1972; Krantz, 1970; Farant et al., 1973; Cotton and Dosman, 1978a and b; Chan-Yeung et al., 1978).

The concentration of suspended dust may be very high. In one study of Canadian grain elevators, Farant and Moore (1978) reported levels ranging from 0.18 to 781 mg/m^3 of air. In spite of the fact that many existing grain elevators are old and lack adequate dust control equipment, the technology does exist to control dust to a short-term mean average concentration for total dust of 10 mg/m^3 (Farant and Moore, 1978).

This dust may become suspended in the air as grain is harvested, transported, moved from one container to another, or cleaned. Dust that settles in work areas may be resuspended through the activities of workers. Therefore, people working on farms and in storage and transport facilities often may inhale grain dust.

Prolonged exposure to grain dust has long been associated with respiratory disorders. Ramazzini (1713), early in the Eighteenth Century, described conjunctivitis, rhinitis, cough, shortness of breath, and cachexia as being common among grain sifters and measurers. Blackley (1880) incriminated the spores of Penicillium as sensitizing agents in severe bronchitis following inhalation of air from Penicillium-infested straw. Wittich (1940), in his discussion of various grain mill dust allergens suggested that smut allergy and grain dust allergy were closely related, but did not have the same allergens. Fawcett (1938), Harris (1939), and Ordman (1958), also implicated fungi associated with cereals and present in cereal grain dust as the cause of respiratory and skin allergies in workers handling cereals. Since then, studies have sought to determine the composition of grain dust, to describe the effects that grain dust has on lungs and the general health of those exposed, and to determine the causes of the symptoms.

A. CHARACTERIZATION OF GRAIN DUST

Microorganisms are consistantly present in grain dust including fungi and bacteria of various species. The predominant species of fungi may vary dependent on the history of the grain. The microbial flora in grain elevators is in part a reflection of the type of grain being handled, its original growing conditions, and the prevailing storage practices. Some organisms

contaminate the grain in the field, while others are acquired during storage. Field fungi are those that invade or contaminate the developing or mature seed while it is still on the plant (Christensen, 1957). Major saprophytic genera of field fungi present in non-weathered grain include Alternaria, Fusarium, and Helminthosporium. Weathered seed may also be invaded by Cladosporium, Diplodia, Chaetomium, Rhizopus, and Absidia. Penicillium oxalicum, P. funiculosum, and in the lesser numbers, P. cyclopium, are consistantly isolated from freshly harvested corn (Mislivec and Tuite, 1970). Bacteria often form 90-99% of the microflora of freshly harvested grain (Wallace, 1973). Fifteen plant pathogenic species of bacteria have been associated with cereal kernels. Sixty-four other saprophytic bacterial species have also been detected on cereal grains or their commercial by-products. Some of the more common bacteria detected include Enterobacter agglomerans (= Erwinia herbicola), various species of Pseudomonas, Bacillus, and Streptomyces (Wallace, 1973). However, not all field organisms actually reach the storage facilities. More than 90% of the epiphytic microflora can be expected to be removed during routine grain cleaning operations (Dutkiewicz, 1978a). Additionally, most field fungi do not survive very long after exposure to dry storage conditions. Fusarium and Helminthosporium may not be recoverable from cereals after a few months in storage (Christensen and Kaufmann, 1965). Dust collected behind harvesters contained large numbers of fungal spores that ranged from 3.5 to 200 million/m^3 of air (Lacey, 1980). Commonly isolated genera are listed in Table 1. After storage, genera common before harvest were seldom isolated, while other species often became abundant in the grain and were isolated frequently in large numbers in airborne dust of storage facilities and grain elevators (Whidden et al., 1980; Lacey, 1980).

Storage fungi are those organisms that generally develop on or in grain at relatively low moisture content after being stored for some time (Christensen, 1957). Under normal storage conditions, Aspergillus and Penicillium species then become the predominant organisms. Grain that is moist or has been heated often contains Aspergillus fumigatus, Micropolyspora sp., Thermomonospora vulgaris, Candia sp. and other yeasts, Mucor pusillus, and Absidia spp. The species of Aspergillus common to stored grains include: A. versicolor, A. ochraceus, A. flavus, and A. halophilicus. Penicillium species have often been lumped into one group rather than being counted individually because of the difficulty in their identification. However, Mislivec and Tuite (1970) identified the species of Penicillium from both freshly harvested and stored corn kernels. The most frequent, consistantly occurring species on stored corn in their studies proved to be P. cyclopium, P. brevi-compactum, and P. viridicatum.

From the few studies available, the microflora of grain dust includes some of the wide variety of microflora of grain, as might be expected. Again, the type and condition of the grain determines in large part the diversity of species present. Williams et al. (1964) isolated Penicillium oxalicum, Mucor sp., and Rhizopus sp. from settled grain dust in elevators handling mainly wheat, oats, or barley. However, Asperillus glaucus was isolated only from those elevators handling mainly wheat, oats, or barley; Penicillium chrysogenum, Aspergillus glaucus, and Mucor racemosus only from those dealing with wheat or barley; Rhizopus nigricans from elevators handling wheat or oats; and Aspergillus fumigatus and Aspergillus repens from those handling wheat. Thus, the species isolated can be expected to differ depending upon the grain being handled.

Similar findings were reported by Farant and Moore (1978) from Canadian grain elevators. The mean, range, and frequency of species encountered were higher if barley was being handled as compared to wheat. They also suggested that seasonal variations in weather affect the number and species of fungi present, as well as increasing the possibility of heating during storage following very wet seasons. Thirty species of fungi in 15 genera were isolated from 113 samples. Penicillium expansum was found in 60% of the samples. Aspergillus repens and Hormodendrum cladosporoides in nearly 45%, and Alternaria in 29%. Ustilago spores occurred in 88% of the samples when barley was handled, but was only in 69% when wheat was handled (Farant and Moore, 1978). Additional differences existed in the numbers of fungus spores detected at various locations within the elevators. The dust control measures and level of activity at the individual sites seemed to affect the dust level and therefore, the number of spores (Farant and Moore, 1978).

In the air of Polish grain elevators, bacteria were the predominate microoorganisms present. Counts average 50 to 100 thousand Enterobacter agglomerans propagules/m^3 of sampled air and 10 to 50 thousand Staphylococcus epidermidis and Streptomyces sp./m^3 of sampled air in the elevators. However, fungi were present, but less numerous. Aspergillus glaucus, Aspergillus fumigatus and 30 other fungi species averaged less than 10,000 spores/m^3 of sampled air (Dutkiewica, 1978a).

In grain-cleaning rooms of mills, 109,000 out of 189,000 viable microorganisms/m^3 of air were E. agglomerans, while dust that had settled on floors or equipment contained 303 million/g. E. agglomerans comprised 75% of all the gram-negative bacteria and nearly 50% of all microoorganisms in the dust. Bacteria were also recovered in the Duluth-Superior study, but less consistantly. Up to 260,000 bacteria and yeasts were isolated from the dust in

Duluth-Superior grain elevators, but some samples contained no bacteria (Whidden et al., 1980).

Toxic chemicals that contaminate grain may also be found in grain dust. Residues of insecticide that had been applied before harvest or during transportation and storage of the grain have been detected in dust from Australian grain elevators (Murray, 1979). Since 1963, all Australian wheat had been treated before storage with insecticides, such as malathion, dichlorvos, fenitrothione or bioresmethrin. Since most insecticide remains on the surface and little penetrates into the endosperm, high concentrations of insecticide accumulated in the dust produced by abrasion of the grain's surface. For example, grain treated with malathion at 8 mg/kg yielded dust containing 150-250 mg/kg. Similarly, 2 to 4 weeks after treating with 6-12 mg/kg of dichlorvos, 2 mg/kg remained on the grain, while dust from this grain contained up to 400 mg/kg and persisted for several months (Murray, 1979).

Grain may become contaminated naturally with other toxic chemicals. For instance, mycotoxins produced by fungi that grow on grain before harvest or during storage could end up in dust produced by abrasion of the grain. Dust was produced experimentally from aflatoxin-contaminated corn during grinding in the laboratory and as it was moved by augers out of a bin into a holding wagon and back again (Burg et al., 1981). It was recommended that precautions should be taken if there was 20 mg of aflatoxin/g of grain.

B. CLINICAL OBSERVATIONS OF THE HEALTH OF GRAIN HANDLERS

Three major respiratory diseases have been attributed to grain dust inhalation: asthma, chronic bronchitis, and grain fever (Cotton and Dosman, 1978a). In the past, silicosis and extrinsic allergic alveolitis (farmer's lung) have also been considered dust related health problems of grain elevator workers.

However, in recent studies relating to health problems of grain industry workers, indications of alveolar damage and fibrosis characteristic of silicosis have been absent (Warren et al., 1974; Davies et al., 1976, do Pico et al., 1977). In fact, only a single confirmed case of silicosis has been reported that involved a grain elevator worker (Heatley et al., 1944).

Farmer's lung, while usually associated with moldy hay, may also be associated with grain that has undergone heating (Dennis, 1971). The symptoms of farmer's lung, which include alveolar damage and diffuse nodules, have also been absent in the most recent studies of grain workers (Warren et al., 1974; Davies et al., 1976; do Pico et al., 1977). Therefore, silicosis and farmer's lung do not now appear to be major problems in the grain workers examined.

The prevalence of grain dust asthma in grain workers has been difficult to estimate. Chan-Yeung et al. (1978) and Warren et al. (1974) have reported both immediate and delayed asthmatic reactions with bronchial challenges of grain dust extracts, but only a relatively low precentage of workers examined had such esthmatic reactions. It has been suggested that those new workers experiencing esthmatic reactions left the job soon after the onset of symptoms to take other less hazardous employment. Therefore, it can probably be concluded that the number of long-time grain elevator workers with job-related asthma is very low, since they really constitute a surviving population (Chan-Yeung et al., 1978). While asthma may not be a major contributing factor to chronic lung disease in the surviving population, asthma attacks are occasionally observed in some workers and could be a potential hazard in others (Cotton and Dosman, 1978a).

Chronic bronchitis constitutes a major occupational health problem in grain industry workers. Surveys indicate that from 1/3 to 1/2 of those grain workers polled complained of a chronic, "productive" cough (Chan-Yeung et al., 1978; do Pico, 1977; Cotton and Dosman, 1978a). Smoking proved to be a major contributing factor to this incidence of chronic bronchitis and bronchial obstruction (Sheridan et al., 1978; do Pico et al., 1977; Cotton and Dosman, 1978a). In one study, chronic bronchitis was 22% more prevalent in smokers than non-smokers who worked in grain elevators (Chan-Yeung et al., 1978).

Other evidence that grain dust is harmful comes from epidemiological studies of grain workers from various parts of the world. In Canada, Warren and Manfreda (1980) questioned grain handlers about respiratory symptoms and tested pulmonary function. The same 51 grain workers were examined twice, six years apart. Control workers were studied only the second time. The prevalence of chronic respiratory symptoms changed little in the six years, but was greater in grain workers than controls. Dyspnea (shortness of breath) occurred in 51% of the grain workers and grain fever (a delayed, noctural fever) in 27%. These results were also evident in another longitudinal study where both Canadian grain workers and civic workers were studied two and a half years apart (Chan-Yeung et al., 1981). Each time the prevalence of respiratory symptoms was greater in the grain workers than in the civic workers. Similar findings were reported in one-time studies of grain workers in the United States and Egypt (Rankin et al., 1979; Moselhi et al., 1979).

Grain workers and non-grain workers also differed significantly in their pulmonary functions. The prevalence of airway obstruction was markedly different as measured by the ratio of forced expiratory volume at 1.0 second and forced vital capacity (FEV_1/FVC) (Warren and Manfreda, 1980). When grain workers were tested for a second time six years later, seven grain workers

(14%) had abnormal FEV_1/FVC values both times, seven (14%) had abnormal values both times, seven (14%) had abnormal values only the second time, while 37 were normal both times. Control workers were studied only once. In the other group of Canadian grain workers examined twice (Chan-Yeung et al., 1981), the expected decrease in pulmonary function due to increasing age was more rapid in grain workers than in civic workers each time. Airway obstruction was also more prevalent in grain workers in the United States than in city worker controls (Rankin et al., 1979).

Smoking seems to be a major risk factor in producing both respiratory symptoms and lung dysfunction in grain workers. Non-smoking workers also exhibit adverse changes due to grain exposure (Dosman et al., 1981). For instance, the prevalence of chronic bronchitis was greater in non-smoking grain workers (23%) than in non-smoking control workers (3%) and similar differences have also been found in other studies in Canada and the United States (Chan-Yeung et al., 1981; Rankin et al., 1979). Respiratory symptoms were more prevalent in both groups of grain workers than in either of the smoking or non-smoking controls (Warren and Manfreda, 1980). The increased risk of chronic bronchitis was calculated to be greater from grain handling (a factor of 4.4) than from smoking (a factor of 2.9) (Rankin et al., 1979). In contrast, lung function was affected by grain handling at the same or smaller magnitude than by smoking in one study (Rankin et al., 1979). In an Egyptian study airway obstruction produced by grain handling was believed to be more harmful than that produced by smoking and even to potentiate the effects of smoking (Moselhi et al., 1979).

Grain fever, another major occupational health problem, is a febrile illness associated with dyspnea that occurs several hours after the grain

workers leave the work environment (Chan-Yeung et al., 1978). Additional symptoms, such as an increased prevalance of grain fever after time away from work, have also been attributed to grain fever. Such symptoms indicate a similarity to metal fume fever and byssinosis (Skoulas et al., 1964). However, these latter symptoms have not been observed by other investigators (do Pico et al., 1977). The occurrence of grain fever is rather variable. In one study, Williams et al., 1964, reported an overall incidence of about 6% of grain elevator workers; later studies, indicated incidences varying from 19% to 40% (Muica and Teculescu, 1977; do Pico et al., 1977). The exact cause of grain fever is unknown, but is currently the topic of intensive research (do Pico et al., 1977).

Direct irritant effects, toxic reactions, and allergic responses have been suggested as the three causal mechanisms for occupational lung diseases observed in grain workers. Irritant receptors have been demonstrated in airway mucosa, which respond to stimulation by increasing mucous secretions (do Pico, 1979). Chronic non-specific stimulation of these receptors by grain dust may play a role in the pathogenesis of grain dust disease (Cotton and Dosman, 1978a). Alternatively, these changes may be due to a toxic reaction involving the complement system. Grain dust has been demonstrated to activate complement by the alternate pathway in vitro (Olenchock et al., 1978b). Complement activation by grain dust was shown to be dose dependent and this suggested a capacity to incite inflammatory sequelae in the lung, which could result in the respiratory problems in grain workers. Additionally, endotoxin has been detected in grain dust, which might also contribute to complement activation (Olenchock et al., 1978a).

Toxic compounds such as residual pesticides, grain fumigants, and mycotoxins may also play a role in these problems, but their role has not been elucidated (Cotton and Dosman, 1978b). The effects that mycotoxins produce in

people who inhale small doses are undetermined. However, there have been a few reports of medical problems in people after exposure to agricultural dust containing large numbers of fungal spores and perhaps large quantities of mycotoxins. For example, one group of workers unloading moldy grain from railcars for four hours subsequently developed malaise, headache, stinging bitterness in the mouth, pain in the eyes, chills, fever ranging up to 39.5°C, cough, quickened pulse, and dry, whistling rales in the lungs (Samsonov, 1960). Symptoms lasted four to seven days. Spores of Mucor, Aspergillus, and Pencillium species were isolated from sputum of workers and the air of the workplace, but no mycelium was isolated from their sputum. Therefore, invasive growth of the fungi was not believed to be responsible for the symptoms.

Emanuel et al. (1975) reported illness in farmers cleaning out moldy silage from silox in Wisconsin and termed it pulmonary mycotoxicosis. The farmers suffered burning eyes, nose, throat, and chest immediatedly after exposure to massive concentrations of spores followed by chills, fever, and a dry, irritating cough during the night. Lung biopsy cultured on blood agar yielded five different fungi, including Fusarium and Penicillium species. Histological examination of the biopsy tissue revealed a multifocal, acute process with most bronchioles filled with an exudate of neutrophils and many histiocytes. Two children of one farmer were playing near the silo as he cleaned it and also became ill with fever and cough, but for only a short time. The symptoms thus appeared to be dose-dependent.

Inhalation of Brazilian peanut meal contaminated with aflatoxin has been linked with two deaths caused by pulmonary adenomatosis (Dvorackova, 1976). In one analysis for alfatoxin, lung tissue yielded a blue fluorescent spot at the same R_f as aflatoxin B_1 that also turned yellow after spraying with 50% sulphuric acid as does aflatoxin. Aflatoxin was also implicated in the deaths

of two newborns from Reye's syndrome (encephalopathy with fatty degeneration of the viscera) after both mothers had handled contaminated foders throughout their pregnancies (Dvorackova et al., 1977). Reye's syndrome was also diagnosed as the cause of death of two children, aged eight and twelve months, whose mothers worked at a poultry farm where there was an outbreak of aflatoxicosis (Dvoackova et al., 1977). Aflatoxin B_1 was detected in the livers of both newborns and also in the liver of the one older child for which there was a specimen.

Some evidence exists that indicates allergic responses may produce grain fever and asthmatic-like reactions in grain workers. Bacteria have been implicated in allergic responses in grain workers. Polish grain handlers with respiratory symptoms showed a higher incidence of positive precipitin and skin reactions to Enterobacter agglomerans (= Erwinia herbicola) than asymptomatic workers (Dutkiewicz, 1978b). Tests with Micropolyspora faeni and Aspergillus fumigatus gave much fewer positive reactions. Since a highly significant correlation existed between skin reactions to grain dust and E. agglomerans, it should be considered a factor that increases the risk of respiratory problems in grain handlers.

Grain workers have been tested for allergic response to numerous fungi. Workers harvesting grain may be exposed to as many as 20 million fungal spores/m^3 air (Darke et al., 1976). Positive precipitin reactions were present in 64% of those workers tested, 43% of these also responded with positive skin reations. Extracts from Paecilomyces fabrinosis, Aphanocladium album, and Verticillium lecanii caused reactions most frequently. When workers with skin reactions inhaled extracts of each individual fungus and a mixture all three, all felt a tightness in their chests and their FEV_1 significantly decreased

(Darke et al., 1976). Commercial extracts of Alternaria, Aspergillus, Candida, Fusarium, Penicillium, yeast, rust, and smut, but produced few, if any, positive skin reactions on grain workers (Warren et al., 1974; do Pico et al., 1977; Chan-Yeung et al., 1979). However, Skoulas et al. (1964) found 73% of grain handlers with respiratory symptoms had positive skin reactions to a mixed extract of Aspergillus, while 36% without symptoms also reacted; 43% with symptoms reacted to mixed Penicillium extracts, but only 14% without symptoms reacted. With the exception of the study by Drake et al. (1976), extracts have apparently not been made from species of fungi actually isolated from the work environment. A mixture of several fungus species in genera common to cereals may not actually contain the antigens to which the workers were exposed. Further tests need to be conducted using fungi isolated from the grain worker's particular environment in order to determine the role of fungi in grain dust disease.

EXPERIMENTAL STUDIES ON GRAIN DUST AND ITS CONSTITUENTS

The effects of grain dust for eight hours a day, five days a week for 16 weeks or continously (three 8-hour periods) for two to 28 days developed clusters of macrophages in the alveolar sacs and alveoli, but no fibrosis (Armanious et al., 1982). Total dust levels were 5.4 g/m^3 after 30 minutes and deceased to 1.2 g/m^3 after eight hours. The respirable portion of the dust (<10 um in diameter) was only 4% of the total dust and part of that 4% cannot penetrate into the lower respiratory tract of mice. The quantity of dust actually inhaled was difficult to assess, especially when a massive proportion of particles were of a size that would clog the nasal passages of mice.

Crude extracts of grain dust were inhaled by human volunteers in doses of up to 5 ml of a 1/10 dilution of the extract (Warren et al., 1974). Either immediate or both immediate and late respiratory symptoms developed in seven of the eight subjects with skin hypersensitivity to grain dust, but not in the

controls. However, systemic symptoms, such as malaise, headache, and fever, were not correlated with positive skin reactions to grain dust. It seemed that although immunological reactions were responsible for the respiratory symptoms, they were not responsible for the systemic symptoms. Different results were obtained with 11 grain workers in the United States. Respiratory response to durum wheat dust extract was unrelated to skin hypersensitivity and there were no systemic reactions (do Pico et al., 1981; 1982). Sodium cromoglycate inhibited the bronchial response to the dust extract and histamine or another mediator was postulated to appear in response to direct irritation. Another mechanism suggested was nonspecific bronchial reactivity, but constituents of the dust that might be responsible have not been determined.

Attempts were made to duplicate in animals the febrile syndrome described by Samsonov (1960) in workers unloading moldy grain from railcars. Guinea pigs were exposed to an aerosol of spores from Dendrodochium toxicum, Stachybotrys alternans, and Aspergillus niger (Samsonov and Samsonov, 1960). Histological examination of the lungs revealed local serous inflammation, necroses, and ulcerations, while bronchopneumonic foci occurred in the pulmonary parenchyma. Atelectasis was observed with compensatory emphysema and there was no evidence of hyphae from a mycotic infection. the authors suggested that a toxic agent in the spores was responsible for he observations in both the guinea pigs and the workers (Samonov and Samonov, 1960). No attempt was made to isolate and administer the toxic agent to reproduce the syndrome. An immunologic reaction has also been suggested as the causative factor of these symptoms (Austwick, 1983). Thus, the etiology appears to be in doubt.

D. Objectives of the Cooperative Agreement

This report contains a summary of the work performed under a cooperative agreement between Tulane University's School of Public Health and Tropical

Medicine and U.S. Department of Agriculture's Southern Regional Research Center. Publications resulting from the research are included and provide more details of the data obtained. Briefly, the purposes of the study were to determine the constituents of grain dust including fungi, bacteria, endotoxins, pesticides, and mycotoxins, and to expose laboratory animals to the mycotoxins that were found in grain dust and determine the extent of their effects, especially on immune function.

The project was arranged to facilitate the interaction of the researchers from both SRRC and Tulane. Consequently, most research was conducted with the participation of both SRRC and Tulane researchers. While data included in this report describe the work performed on the cooperative agreement by Tulane, the inclusion of data from what was a majority effort on the part of SRRC researchers was considered appropriate because of the contribution from the Tulane investigator.

II. CHARACTERIZATION OF GRAIN DUST CONSTITUENTS

A. Dust Sample Collection

The participation of five grain elevator/transfer facilities along the lower Mississippi River was recruited for this project. There were two types of grain dust samples collected, respirable dust and settled dust. Respirable dust (10 μm or less in aerodynamic diameter) was collected with a Bendix personal air sampler pump on a preweighted membrane filter (0.45 μm pore size). Dust larger than respirable size was prevented from reaching the filter by placing a plastic cylone before the filter. Respirable dust samples were stored at 4°C. Settled dust was collected as grab samples by scooping up the dust from the floors, equipment, ledges, or dust control systems. Settled dust samples were sifted through a 40-mesh screen (420 μm openings) to remove large

debris before being stored at 4°C. A total of 174 respirable and 60 settled grain dust samples were collected from six grain elevators during the four-year project.

B. Microflora and Endotoxin Analyses

Samples for the microflora analyses were collected in three phases, (1) a preliminary phase in which 33 respirable dust and 12 settled dust samples were collected from two different terminal grain elevators, (2) an expanded sampling phase in which five elevators were sampled, collecting a total of 69 respirable dust and 20 settled dust samples, and (3) a seasonal sampling phase in which only two elevators were sampled eight times during the year at least six weeks apart. A total of 72 respirable dust and 28 settled dust samples were collected in the third phase.

Respirable dust and settled dust samples were analyzed for the quantity and type of aerobic, saprophilic, mesophilic fungi and bacteria. Half of a dust-laden filter from the personal air sampler (respirable dust sample) was placed in a sterile test tube with 20 mL of a sterile, 0.1% aqueous solution of Tergitol ND-10®, a surfactant. In the case of settled dust, 0.5 g of the sieved dust was placed into a sterile test tube with 20 mL of the sterile tergitol solution. After agitating well with a vortex mixer, serial dilutions were prepared and placed on four replicate agar plates. Initially, three agar media were used for enumeration of fungi: (1) Czapek's agar with aureomycin, Tergitol NP-10®, and corn steep liquor, CS agar; (2) potato dextrose agar, PDA; and (3) malt salt agar, MS (Whidden et al., 1980). CS and PDA favor the growth of saprophytic fungi, such as Aspergillus and Penicillium. In later studies, only CS was used to enumerate saprophytic fungi because PDA did not support such compact growth as did CS and because PDA was not providing any additional

information. Plates were incubated at 25°C for 7 days. The number of colonies were recorded and identified to species where possible.

Aliquots of the serial dilutions were placed on four replicate agar plates for viable counts of bacteria. The medium used for total plate counts was tryptone, glucose, yeast-extract agar, TGY (DeLucca et al., 1982), for isolation of Bacillus thuringinesis, nutrient agar with penicillin and polymixin, NPP (Saleh et al., 1969), and for enumeration of Gram-negative bacteria, Endo agar (Difco, Detroit, MI). All bacteriological plates were incubated at 30°C for 48 hours. Representative colonies were isolated for identification after the colonies on the plates were counted.

The remaining half of the filter was treated as described by De Lucca et al., (1984) for determination of the endotoxin content in the respirable dust. Portions of the sieved settled dust were also analyzed for endotoxin.

In the preliminary study, Aspergillus and Penicillium were the most frequently isolated organisms. The range and mean for the two elevators are listed in table 3. Species that were encountered frequently included the following: Aspergillus terreus, Aspergillus fumigatus, members of the Aspergillus flavus group, Penicillium corylophilum, Penicillium cyclopium, Penicillium puberulum, and Penicillium viridicatum. Few Fusarium colonies were isolated, but when they were isolated, they were Fusarium moniliforme (Palmgren et al., 1983). Respirable dust samples contained fungi similar to the settled dust, but some of the fungi with larger spores, i.e. Cladosporium, Alternaria, were not isolated as often as from settled dust.

The fungi were of similar number to those detected in grain dust of Duluth - Superior elevators, but the species were different (Whidden et al., 1980; Palmgren, 1985). The maximum respirable dust concentration found was 1.22 g/m^3 and was unrepresentative of the usual concentrations. The worker wearing the

sampler had to go into the baghouse of the dust control system to correct a malfunction. The person was covered with dust when he emerged. Consequently, the sample was not representative of a routine dust exposure nor did it represent that worker's exposure since adequate respiratory protection was worn. As would be expected the highest number of spores was in this sample, $1x10^{10}$ colony forming units (CFU) per m^3 of air. The next highest concentrations were 371 mg of dust/m^3 of air and $5.5x10^6$ CFU/m^3 of air (Palmgren, 1985).

The second phase of the study included samples from five elevators. No statistical differences were observed when the number of fungi in samples from different elevators were compared nor in samples from people in different job classifications. Aspergillus and Penicillium were the most commonly occurring fungi.

The mycoflora in the samples collected in the third phase appear to differ from the previous studies in the species of Aspergillus isolated. Aspergillus flavus and/or Aspergillus parasiticus appeared to be more common in the samples from the third phase than the earlier phases. Much corn being shipped was said to be that received as part of the federal "Payment - In - Kind" (PIK) program. The elevator personnel said that the quality of some PIK corn had been lower because of the longer time it had been in storage. One bargeload had been refused and shipped back up the river while we were sampling because of the musty, weathered condition (fungal damage) of the grain. However, before sending it back, the grain had to be unloaded, weighed, and reloaded on the barge. Handling of the grain did result in a large number of fungi in our samples that day. Statistical analyses of the mycoflora data are being conducted.

In the respirable dust samples from the preliminary study, few (13%) of the samples contained any bacteria and the concentrations were low Bacillus thuringiensis was recovered from only 3 of 54 respirable dust samples. The most prevalent variety was B thuringiensis var. aizawai, which is not one of the common soil varieties nor the commercially available variety. (Palmgren et al., 1983) This trend continued in the second phase. The variety aizawai was nearly 95% of those recovered. More settled dust, samples contained B. thuringiensis, 55%, than respirable dust samples, 17% (DeLucca et al., 1982). A previously undiscovered variety was isolated from grain dust and named B. thuringiensis var. colmeri (DeLucca et al., 1984).

Bacterial analysis revealed that settled grain dust had a high population of bacteria that ranged from 1.9-53.4 million/g for total plate count, 0.9-14.8 million/g for gram positive bacteria, and 0.1-50.0 million/g for Gram - negative bacteria (GNB). In all but one of the samples, GNB were the most prevalent. Although settled dusts contained large populations, only four of the airborne samples had countable populations ranging from 20,000 to 90,000 per filter for both total plate and GNB counts (DeLucca et al., 1984).

Two hundred and thirty GNB were isolated and identified. Enterobacter agglomerans was the most predominant with 61.3% (141 of 230) of the total. Other bacterial genera found were: Pseudomonas (9.1%), Serratia (6.9%), Acinetobacter (6.9%), Klebsiella (3.9%), other Enterobacter species (3.5%), and various genera (8.4%) (DeLucca et al., 1984). The finding of E. agglomerans as the predominant species agrees with data from Polish grain dusts (Dutkiewicz, 1978a); it is also present abundantly in cotton, flax and in the air of industries processing these materials (Morey et al., 1983; Rylander and Morey, 1982).

Analysis of settled grain dust showed a much greater amount of endotoxin present than in the airborne samples. The settled dust samples had endotoxin levels ranging from 22.5 to 187.5 ng per mg of dust. Most of the respirable dust samples, 62% (43 of 69) did not contain detectable levels of endotoxin, while 33% (23 of 69) assayed had 0.6 ng and 3 samples (5%) had 6.0 ng. Dust collected on the membrane filter of the air samplers ranged from 0.002 to 91.8 mg during the 8-hour collection period. The volume of air sampled during this period was 0.81 m^3. Two of the respirable samples contained much higher endotoxin concentrations than the other samples, 39.2 and 25.7 ng/mg. The average for the remaining 24 samples was 2.9 ng/mg. Therefore, the endotoxin concentration ranged up to 7.4 ng/m^3 of air (DeLucca et al., 1984).

In this survey (DeLucca et al., 1984), the endotoxin values obtained for the settled dust samples were greater than a previously published report on levels in respirable and settled grain dusts (Olenchock and Major, 1979). The respirable samples contained 331.2-439.5 ng/g, while the sole settled sample contained 429 ng/g. However, settled dust endotoxin values are not as high as those found in airborne dusts from flac and cotton mills, and from airborne dust from non-textile industries (Rylander and Vesterlund, 1982; Cinkotai et al., 1977).

The respirable dust samples collected in the third phase had no viable bacteria. However, endotoxin concentrations, which ranged from none detected to 6.4 ng per 8-hour sample, indicated that viable Gram-negative bacteria (GNB) had at some time been associated with the respirable dust or the grain from which the dust was generated. In settled dust samples, the number of bacteria from both total plate counts and GNB counts remained relatively stable, but the genera of GNB varied. Enterobacter agglomerans was the predominant species in warm months, while Pseudomonas sp. and Klebisella sp. increased in the winter

months. Citrobacter sp. and Serrtia sp. were also recovered. Endotoxin concentrations in settled dust samples were high, ranging from 0.3 to 5.6 ng/mg of dust (DeLucca and Palmgren, in review).

C. Mycotoxin Analyses

1. Analyses of Grain Dust

There were three separate phases of sample collection for mycotoxin analyses of grain dust:

First, a preliminary sampling of 2 elevators were conducted. Fifteen samples were collected consisting mostly of corn, but also some soybean dust.

Second, an extended study of five elevators was conducted. Thirty-four settled dust samples were collected again mostly corn, but some soybean and wheat dust.

Finally, a seasonal study of two elevators with repeated sampling was conducted throughout one year. This time dust was collected only when corn was being shipped. Twenty-eight samples were collected on eight sampling dates. Each date was at least six weeks apart from each other.

All dust for mycotoxin assays was collected from accumulations on floors or equipment and from dust control systems. Prior to extraction, the settled dust samples were passed through a 40-mesh sieve to remove any large debris that may be present. Respirable dust samples were too small to be analyzed for mycotoxin.

A 25-g portion from each of the settled grain dust samples was mixed with 250 mL of methylene chloride (CH_2Cl_2), 25 mL of water, and 25 g of Celite. This mixture was shaken for 30 minutes on a wrist-action shaker and then filtered through sodium sulfate (Eppley et al., 1968). Three 50-mL portions of the filtrate were collected. The first 50 m L-aliquot was examined for aflatoxins by eluting the extract from a silica gel column as described in the

CB method (AOAC, 1980). Whenever chloroform was specified in the CB mehtod, methylene chloride was substituted. There was no difference between the two solvents for this purpose (Lee and Catalano, 1981). The CB column was washed sequentially with 150 mL of hexane, then 150 mL of anhydrous diethyl ether. The aflatoxins were eluted with CH_2Cl_2:CH_3OH (19:3,v:v). The elutant from the column was evaporated and reserved for aflatoxin quantitation by thin-layer chromatography (TLC) and fluorodensitometry.

A second 50-mL aliquot of CH_2Cl_2 extract was shaken with 100 mL of a 5% solution of sodium bicarbonate. Thew CH_2Cl_2 was discarded, while the aqueous layer was collected. Ten milliliters of formic acid and 90 mL of CH_2Cl_2 were added to the aqueous layer and the mixture was shaken. The aqueous layer was discarded and the CH_2Cl_2 layer was washed with 100 mL of water and reserved for ochratoxin analysis by TLC (Paulsch et al., 1982).

A third 50-mL aliquot for the CH_2Cl_2 extract was shaken with 50 mL of a 7.1% aqueous solution of citric acid and 50 mL of benzene. The benzene layer was collected and saved. The aqueous layer was shaken with an additional 50 mL of benzene. The aqueous layer was discarded and the benzene layer was combined with the benzene layer from the previous step and reserved for zearalenone analysis by TLC (Ware and Thorpe, 1978).

The solvent was evaporated from the solutions under N_2 and the residue reserved for TLC. Each residue was dissolved in 0.2 mL of CH_2Cl_2 and 1-5 µL spotted on TLC plates. Aflatoxins, ochratoxin, and zearalenone were quantitated by standard TLC methods.

For secalonic acid D (SAD), a separate extract was prepared according to the method described by Ehrlich et al., (1982). Twenty g of settled dust was combined with 100 ml of ethyl acetate and mixed intermittantly for one hour.

The suspension was filtered and the first 50-ml of filtrate was collected. The extract was concentrated on a rotory evaporator to a volume of approximately one ml. The concentrated extract was added to 10 ml of cold hexane and allowed to stand one hour in an ice bath before centrifuging at 2000 rpm. The supernatant was discarded and the precipitate was washed with 10 ml of cold hexane. Again the supernatant was discarded. The precipitate was redissolved in one ml of acetonitrile and centrifuged. The precipitate was discarded and the solvent of the supernatant was evaporated. At this point, the samples could be analyzed either by TLC and only the positive samples quantitated by HPLC or directly by HPLC.

TLC plates were developed in benzene: ethyl acetate: formic acid (10:4:1) and then sprayed with $FeCl_2$ solution. For quantitation of SAD, 20µl of samples were injected into the HPLC with a C_{18} reverse phase silica column. The solvent was acetonitrile: water acetic acid: tetrahydrofuran (40:30:5:5) flowing at a rate of 1.7 ml/min. The fluorescent intensity at 340 nm for the sample was compared to that of a standard solution of SAD.

In the preliminary study of grain dust from two elevators, the dust was analyzed for aflatoxin, ochratoxin, and zearalenenone, but not SAD. No aflatoxin was detected. nor was ochratoxin.

However, 67% of the samples contained detectable levels of zearalenone. In one elevator, 42 ng/g was the average for zearalenone-positive samples and 36 ng/g for the other elevator (Palmgren et al., 1983).

In the second study of five elevators, dust was analyzed for aflatoxin, ocharatoxin, zearalenone, and SAD. Only two samples contained aflatoxin and at low levels. No ochratoxin was detected. However, 65% of the samples had SAD and 68% had zearalenone. Prehaps more important was the finding that 41%

contained both SAD and zearalenone, while one sample contained all three toxins. Only three samples did not have detectable levels of at least one mycotoxin. That means 91% of the samples had detectable levels of at least one of the four mycotoxins.

In the last study, two elevators were sampled repeately. Dust was analyzed for aflatoxin and zearalenone. No samples were analyzed for ochratoxin, because none was detected in the previous samples. Time on the project ran out before the samples could be analyzed for SAD. A surprising result was that 71% of the samples contained detectable concentrations of aflatoxin B_1. However, zearalenone was detected in only 18% of the samples. One sample contained both aflatoxin and zearalenone. This particular year, the results were nearly opposite of those in the two previous years.

These studies indicated that more than one mycotoxin frequently occurs in grain dust. In addition, the predominate mycotoxin was not necessarily the same each year, as would be expected from the sporactic nature of mycotoxin occurrence in the field. However, we had not expected to find the relatively high frequency with which these mycotoxins were detected in settled grain dust.

2. Analyses of Fungal Mycelium and Spores

Two distinct reservoirs of mycotoxins exist in fungal-infected cereal grains - the fungal spores and the spore-free mycelium-substrate matrix. Many fungal spores are of respirable size, while the mycelium-substrate matrix can be pulverized to form particles of respirable size during routine handling of grain. In order to determine the contribution of each source to the level of mycotoxin contamination of dust, we inoculated 100 g of autoclaved rice at 42% moisture level with a color-mutant of Aspergillus parasiticus or one of four fungi isolated from grain dust, Aspergillus parasiticus (SRRC - 2004),

Aspergillus niger (SRRC - 2005), Aspergillus fumigatus (SRRC - 2006), and Penicillium oxalicum (SRRC - 2007). The cultures were incubated for seven days at 25°C and then dried in an oven at 50°C for three days.

The spores were separated from the mycelium by shaking a 10-g aliquot of the culture and collecting the airborne spores on a membrane filter (pore size 0.45 μm). A weighed aliquot of spores were counted in a hemacytometer chamber. The remaining spores were disrupted by glass beads in a Braun homogenizer before being extracted with methylene chloride. The mycelium - substrate matrix (MS matrix) was collected after the spores were blown off, weighed and extracted with methylene chloride. Another 10-g portion of the untreated culture was extracted with methylene chloride. This portion contained both spores and the MS matrix.

Extracts were made to a known volume in benzene:acetonitrile (98:2) and chromatographed according to standard procedures for each toxin. Aflatoxin B_1 from the A. parasiticus culture, norsolorinic acid from the mutant of A. parasiticus, fumigaclavine C from the A. fumigatus culture and naptho-α-pyrones from the A. niger culture where quantitated by TLC according to standard procedures (Pons, 1968; Lee et al., 1971; Cole et al., 1977; Ehrlich et al., 1984). Secalonic acid D from Penicillium oxalicum was quantitated by HPLC (Reddy et al., 1981).

Conventional quantitiative chromatographic analyses of separated materials indicated that aflatoxin from Aspergillus parasiticus, norsolorinic acid from a mutant of A. parasiticus, and secalonic acid D from Penicillium oxalicum were concentrated in the mycelium-substrate matrices and not in the spores. In contrast, spores of Aspergillus niger and Aspergillus fumigatus contained

significant concentrations of aurasperone C and fumigaclavine C, respectively; only negligible amounts of the toxins were detected in the mycelium-substrate matrices of these two fungi.

Previous reports of toxin concentrations in spores did not relate the number of spores/g to the toxin levels for a given number of spores. Our results provide information on the number of spores/g and toxin levels for a given number of spores (Table 4). Since spore counts are a routine part of dust analyses, our data are necessary to determine the magnitude of toxin contamination as related to spore counts. Since aflatoxins are produced by A. parasiticus and are amoung the most potent carcinogens, both the distribution of aflatoxin B_1 between spores and the MS-matrix and the content of this toxin in spores would be important. While 10^6 spores contained just 1 ng of aflatoxin B_1, the A. parasiticus MS-matrix contained 16-times that amount.

Clearly, when mycotoxins are contained within spores, a much greater probability of exposure for workers handling moldy grain would be expected because the spores are more easily suspended in the air and remain suspended for long periods of time. The mycotoxins retained in the MS-matrix would pose a problem when the MS-matrix becomes pulverized. For toxins present in the MS-matrix, secalonic acid D and aflatoxin, the larger quantity of toxin was in the MS-matrix and could pose a considerable hazard if dust were produced from heavily contaminated grains (Palmgren and Lee, in press).

D. Pesticide Analyses

Corn, soybean, and wheat dusts were analyzed for two pesticides, malathion and diazinon. Malathion was applied to grain at grain elevators whenever the purchaser requested it and probably at some time prior to delivery to the terminal grain elevator. Diazinon was a common field pesticide for midwestern crops.

Dust was spiked with pure standards of both pesticides prior to assay of the dust samples. To a blended sample of corn dust, known amounts of malathion or diazinon (0-50 µg/g) were added to determine the efficiency of subsequent extraction and analytical procedures. These spiked dust samples and the 31 grain dust samples collected from the grain elevators were extracted with acetone as recommended by an FDA method for determination of organophosphorus residues in nonfatty food and feed samples. However, grain dust samples were quite dry, so that steps requiring extraction from an aqueous layer and drying with sodium sulfate were not necessary. The extraction procedure was carried out in glassware cleaned with nitric acid and high pruity methylene chloride. Distilled-in-glass solvents were used throughout.

The procedure was as follows:

1. Shake 5 g of grain dust with 10 mL acetone for ten minutes in glass vial having Teflon-lined cap.
2. Filter suspension through Millipore AP4003705 glass fiber filters under vacuum.
3. Rinse extraction vial with 5 mL methylene chloride and pour through filter.
4. Rinse funnel and filter with an additional 5 mL of methylene chloride.
5. Transfer filtrate to a 125-mL evaporating flask.
6. Rinse vacuum flask with 5 mL of methylene chloride and add to evaporator.
7. Add boiling chips rinsed in methylene chloride.
8. Using rotary evaporators, evaporate under vacuum with mild heating to a volume of 1.5 of 2.0 mL.
9. Transfer to 2 mL glass vial having Teflon-lined septum cap.

The concentrated extract was analyzed directly without benefit of a Florisil or other clean-up step. No deterioration of the chromatographic columns or other difficulty resulted from the omission of a clean-up step.

The insecticide concentrations were determined by gas chromatography using a Hewlett-Packard 5710A chromatograph equipped with a thermionic nitrogen-phosphorus detector. Initial determinations were made using a 10% OV-101 Chromosorb W H-P 100-120 mesh support, 6-foot glass column with a 3.5 mm I.D. For samples where malathion or diazinon was detected, presence of the insecticide was confirmed on a second column composed of 1.5% SP-2250 and 1.95% SP-2401. Flow rates were 40 mL/min for the nitrogen carrier gas, 50 mL/min for air to the detector, and 3 mL/min for hydrogen to the detector. The temperature for the injection port and detector was 250°C. The column temperature was 210°C. The volume of sample injected was 5 µL. Chromatograph response was calibrated by injection of 2 to 10 µL of standard malathion and diazinon solutions in trimethylpentane.

Determining the efficiency of the extraction and analytical procedure for malathion was complicated by the presence of malathion in all samples collected. Because of this, the recovery efficiency at the lowest concentration of malathion, 0.1 µg/g was low; therefore, concentration in that range were not accurate. Recovery of malathion was much better between 1 and 50 µg/g of dust with the mean recoveries ranging from 83 and 92%. Diazinon was recovered over the range of 0.1 and 50 µg/g with mean recoveries of 74 to 91% (Palmgren and Lee, 1984).

Of the 31 grain dust samples, all had detectable concentration of malathion; none had detectable concentration of diazinon. The highest concentration of malathion detected was 32 µg/g, while four others exceeded

µg/g and the mean concentration was 6 µg/g (Palmgren and Lee, 1984).

Australian insecticide residue limits reflect a difference in distribution, such that the residue limit for whole grain is lower than that for dust (Murray, 1979). Levels of malathion in grain, for example, may be at the maximum residue limit of 8 µg/g, while dust obtained from that grain would have residue in the range of 150-250 µg/g (Murray, 1979). The level in dust was 18 to 30 times greater than that in the whole grain. Such an increase in concentration of insecticides in grain dust could post a hazard to humans who might inhale it, or especially animals who might ingest large quantities of pelleted grain dust.

While no limits or tolerances have been established for malathion in grain dust in the United States, a limit of 50 µg/g was established for the maximum allowable malathion residue in citrus pulp when used for cattle feed. This level is comparable to those established by the Australian government for malathion residues in grain and grain dust. Australian limits for malathion in feeds, including pelleted grain dust, varied from state to state, but were commonly set at 100 µg/g, (Murray, 1979). The levels of malathion detected in our study were well below that limit, ranging from 0.17 to 32 µg/g dust. Animal feed quality would be within prescribed limits for Australian standards and those established for citrus pulp.

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for malathion was set at 15 mg/m^3 of air (Fairchild, 1977). Even at highest level of malathion that was detected, 32 µg/g dust, a worker would have to be exposed to 500 g of dist/m^3 of air to exceed the TLV for malathion. In previous studies, levels of grain dust did not even approach such an extreme. Respirable dust levels previously reported

for Canadian grain elevators, ranged up to 97.3 mg/m^3 in country elevators and up to 76.3 mg/m^3 in terminal elevators, (Farant and Moore, 1978). Terminal grain elevators in the United States had levels of respirable grain dust up to 8.3 mg/m^3 in another study (Whidden et al., 1980) and up to 370 mg/m^3 in another study (Palmgren et al., 1983). These levels, as high as some were, still much below the 500 g/m^3 extrapolated to exceed the TLV for malathion.

Diazinon did not seem to be a problem in grain dust tested in this study. Grain dust samples had no detectable quantities of diazinon. The detection limit was 0.01 µg/g. Despite the relatively low TLV for diazinon of 100 µg/m^3 of air, (Fairchild, 1977) the dust levels would have to exceed 10 kg/m^3 of air for dust containing the lowest detectable level of diazinon (0.01 µg/g of dust) to reach the TLV. Dust levels, as previously reported (Farant and Moore, 1978; Whidden et al., 1980; Palmgren et al., 1983) and discussed, were very much lower than 10 kg/m^3. Therefore, the dust at levels we observed posed no apparent hazard with respect to diazinon.

No tolerances or limits have been established for diazinon residues in animal feed. However, tolerances of diazinon in grain were established at 0.75 µg/g of corn and grain sorghum 0.1 µg/g of soybeans, (U.S.E.P.A, 1980) and 0.05 µg/g of wheat (U.S.E.P.A, 1982). The tolerance in wheat was set at a level very close to the practical detection limit for diazinon in grain dust, if diazinon were to have been detected in wheat dust, that level would have approached the tolerance limit for wheat. From our data, we would not predict any diazinon residue problems for animal feed containing grain dust, unless possible, wheat dust was a constituent and contained a detectable amount.

The problems with excessively high levels of insecticides in grain dust that occurred in Australia (Murray, 1979) were not observed in the grain dust

we examined for malathion and diazinon. However, dust escaping from grain that was being treated, or that had been treated shortly before handling, might transiently contain higher, more hazardous levels of insecticides. Additionally, we analyzed for only two insecticides. Our data (Palmgren and Lee, 1984) does not exclude the possibility that other insecticides or fumigants might be present in unacceptable concentrations.

E. Scanning Electron Microscopy of Grain Dust

Specialized analytical methods are often required for identification of components of agricultural dusts. The larger particles and biological entities can often be identified visually with the aid of light microscopy. However, most of the respirable portion of the dust retains few structural features for identification. Corn and soybean dusts that were examined with scanning electron microscopy and x-ray microanalysis consisted of structurally unrecognizable particles similar to those of cotton dust. In addition, the dusts contained many spheroid particles identified as starch. Dust was found to aggregate forming larger particles. This aggregation could distort instrumental analysis of dust particle size (Goynes et al., in press).

III. ANIMAL EXPOSURE TO SECALONIC ACID D, A GRAIN DUST CONSTITUENT

An animal inhalation chamber was purchased by Tulane School of Public Health and Tropical Medicine to expose laboratory animals to an aerosol of grain dust. However, the dust generating system to be developed by SRRC personnel was not operating except for test measurements by the termination date for the project.

While inhalation exposure to grain dust were not performed, laboratory animals were exposed to secalonic acid (SAD), a mycotoxin frequently detected in grain dust. Isolated, ventilated lung perfusion and tests of immune system

function after intraperitoneal injection of SAD examined the effects of SAD on animals. For both studies, SAD was produced by inoculating Penicillium oxalicum (NRRL 5209) into 300 ml of a 2% yeast extract - 10% sucrose medium in a 2.8-L Fernbach flask. After two weeks of incubation at room temperature, the medium was decanted and discarded. The mycelium was extracted twice with methylene chloride according to the method of Ciegler et al., (1980). The extract was dried with anhydrous sodium sulfate and concentrated by flash evaporation until only an oily residue remained. The oily residue was redissolved in about 1 mL of methylene chloride, added to cold hexane, and stored at 4°C overnight. The yellow precipitate that formed was filtered through a Whatman #4 filter and redissolved in a minimum volume of hot chloroform. The solution was cooled until the SAD recrystallized. Redissolving and recrystallization steps were repeated twice. The final identification and determination of the level of purity of the SAD crystals was ascertained by specific melting point, ultraviolet spectroscopy, and mass spectrometry.

A. Isolated, Ventilated Lung Perfusion

The metabolic activity of lung cells remains high even after several hours of perfusion in isolated, ventilated perfused lungs. By injecting a solution of SAD into the trachea of an isolated, ventilated rat lung, it could be determined if SAD was biologically altered and if it would be transported to the circulatory system.

In this procedure, a male, Wistar rat weighting at least 300g was anesthetized with a 1% mixture of Halothane in 95% O_2 - 5% CO_2. The trachea was cannulated and when the chest cavity was opened, the cannula was attached to a Harvard positive pressure respirator (model 680) at a frequency of 20-30

strokes/min., 3 ml/stroke. The flow rate and percentage of the Halothane mixture were lowered when the mixture was supplied to the respirator. The abdominal artery was severed. The right ventricle was pierced and a polyethylene tube filled with perfusate buffer was inserted through the opening into the pulmonary artery and tied in place. The left atrium was cut, the lung was perfused with perfusate buffer, and the trachea, lungs, and great vessels were dissected free from the connective tissue. The isolated lungs were suspended in a glass, water-jacket chamber maintained at 37°C. The frequency of the respirator was increased to 60 strokes/min., 5 ml/stroke. The halothane mixture was removed and replaced by 95% O_2 - 5% CO_2. The perfusate (37°C) was allowed to flow at 10-15 ml/min for 5 min. before introducing the SAD.

Two methods of introducing the SAD into the lungs were tried. First, crystalline SAD was placed in a glass capillary tube, which was fitted on the end of the polyethylene tubing in the trachea. Air from a syringe was pushed through the capillary tube into the lung. Problems associated with the loss of toxin on the wall of the tubing prohibited accurate delivery determinations. A second method was intratracheal deposition of 0.1 ml of a solution of SAD with a cannula smaller than the inner diameter of the polyethylene tubing in the trachea or by piercing the tubing close to the trachea with a 26 gauge needle on a syringe with the SAD solution. The solution initially contained Krebs bicarbonate buffer: acetone (80:20) (Sorrenson et al., 1982) with 25 mg/ml of SAD, but later was changed to 5% sodium bicarbonate solution: DMSO (9:1) with 2 mg/ml of SAD.

Perfusate fluid was collected for 1 minute prior to instillation of the SAD and 10-20 minutes afterwards at 30 second intervals. Fractions were extracted twice with 2 mL of ethyl acetate. The ethyl acetate was dried with

anhydrous sodium sulfate and evaporated under a stream of nitrogen. The residue was redissolved in 0.1 mL of acetonitrile and analyzed for SAD by the HPLC method of Reddy et al., (1981).

Limited success was achieved with the Krebs buffer-acetone solution. In spite of the lack of pathological effects reported by Sorrenson et al. (1981) when they injected such a solution into the trachea, some immediate damage must have occured, since the perfused lung became edematous and flow of the perfusate was reduced. For this reason, the vehicle was changed to the bicarbonate: DMSO solution.

However, this vehicle is not without drawbacks. The DMSO may be increasing the permeability of membranes for SAD. Small traces of SAD near the limit of detection appeared in the perfusate fluid after passage through the lung. Other methods of administering SAD should be tried to confirm that SAD can cross the membranes without the assistance of DMSO.

B. Immunotoxicity of SAD

There were two phases of the studies to determine if the immune functions of mice were altered. The first phase examined in vivo effects of SAD administration on host resistance to influenza virus infection by determining mortality rate, virus titers in the lungs, and antibody titers to the virus, as well as the histopathology and weights of the lungs, livers, and spleens. In addition, the development of the humoral response was measured by the plaque - forming cell assay, while cell - mediated responses were measured by the response of lymphocytes to mitogen stimulation. In the other phase, the immune functions of inbred mice after SAD injection only were assayed by the plague - forming cell (PFC) assay, antibody formation to sheep red blood cells, and lymphyocyte blastogenesis.

1. Influenza - SAD Interaction

Animals in the first phase were male ICR mice [King Laboratories, Middleton, WI] in groups of 20 weighing between 25 and 35 grams. Mice were housed in plastic cages in groups of five for at least one week prior to treatment. The cages were maintained in a temperature-controlled room (18°C) with artificial illumination (12 hour light/dark cycle). Pelleted feed and fresh water was available ad libitum.

The AiChi (H2N3) influenza virus used in this study was originally obtained from Dr. Richard Berendt, Ft. Detrick, MD, as a M-9, CE-1 preparation (nine mouse and one chick embryo passage). This original virus suspension titered 10-7.2 egg infection doses (EID) per 0.1 ml. The stock virus used in this study was furnished by Dr. Kenneth Soike (Delta Regional Primate Center, Covingtion, LA). The virus titer for M-10, CE-2 passage was 10-7.2 EID and was stored in 1.0 ml-aliquots at -70°C. The stock virus solution was diluted 1:50 in Minimal Essential Medium (MEM). Groups of 20 mice were exposed to the virus aerosol in a modified Henderson apparatus. Instead of a Well's atomizer, a DeVilbis type 40 atomizer aerosolized 5.5 mL of virus suspension for the five - minute exposure. All aerosol exposure procedures were conducted with the aerosol apparatus operating within a Class II, Type B biological safety cabinet.

Five days after virus exposure, SAD was injected intraperitoneally. A dose of 35 mg/kg of body weight was administered in a volume based on 0.1 mL of solution per 30 grams of body weight. The SAD was dissolved in DMSO and diluted in 5% aqueous sodium bicarbonate to yield a final DMSO concentration of 10%. Control mice received 0.1 mL of 10% DMSO in 5% aqueous sodium bicarbonate vehicle solution also five days after virus exposure.

a. Mortality rate

Groups of 20 mice were treated as follows:

1) Exposed to influenza virus aerosol (Flu)

2) Exposed to flu aerosol and injected with SAD five days later (Flu/SAD)

3) Exposed to flu aerosol and injected with vehicle solution (Flu/VC)

4) Injected with SAD (SAD)

5) Injected with vehicle solution (VC)

6) Untreated control (UC)

Animals were checked twice a day for 14 days after flu exposure. The experiment was repeated three times. The numbers of animals dying per group were compared for statistical significance with Chi Square test for two independent variables. No mice died in the UC and VC groups. The daily cumulative mortality data for the other groups are illustrated in Figure 1. Overall, 25% of the SAD, 28% of the Flu, and 82% of the Flu/SAD mice died within 14 days. The Chi square value determined by comparing the mortality in the Flu and Flu/SAD proved that the mortality rates were significantly ($p < 0.001$) different for the two groups.

The harmonic mean time to death for each group was determined and compared with the other groups using the Kruskal-Wassis test. The harmonic mean times to death for Flu and Flu/SAD groups were 8.78 and 8.96 days after virus exposure, respectively. There was no significant difference between the mean times to death.

b. Virus titer in lungs

In order to ascertain viral replication in lung tissues, the lungs were removed from mice exposed to (1) influenza virus aerosols only (Flu), (2) virus

aerosols and SAD (Flu/SAD), (3) SAD only and (4) untreated controls (UC) on 0, 3, 6, 9 and 12 days after aerosol exposure. Four mice from the two groups receiving the virus and two mice from each of the SAD and UC groups were randomly selected at each time interval and sacrificed by cervical dislocation. Lungs were aseptically removed, weighed, and placed into a 10-mL Ten Broek tissue grinder. Lung tissues from each group were pooled. Sufficient MEM with 10% FBS was added to give a final 10% lung suspension. After grinding, the tissue suspension was stored at -70°C, until they were assayed to determine virus titers.

The virus titer of lung tissue suspensions was determined by inoculating ten-fold serial dilutions in MEM into five 10-day old embryonated chicken eggs. Allantoic fluids were harvested after 48 hours and tested for hemagglutination activity. Human type O blood were resuspended at a 0.75% concentration in PBS. In 12 x 75 mm tubes, 0.2 ml of allantoic fluid was mixed thoroughly with 0.8 ml of PBS and 0.5 ml of the red cell suspension. The tubes were incubated at room temperature for one hour and examined for hemagglutination. Those dilutions positive for hemagglutination were recorded and used to determine that dose causing an infection in 50% of the eggs (EID_{50}) by the method described by Reed and Munsch (1934).

The mice in SAD, VC, and UC groups had no viable influenza virus in their lungs. In those mice exposed to the virus, Flu, Flu/SAD, and Flu/VC, the viable virus titer reached a peak three days after exposure to the virus. The mice in the Flu/SAD group exhibited signs of a slower recovery. These signs were confirmed by a higher viable virus titer nine days after virus exposure than in either the Flu or Flu/DMSO groups. However, by 12 days after exposure to the virus, none of the survivors in any of the groups had any viable influenza virus in their lungs.

c. Pathology

Several mice in the SAD group that were moribund or had died recently were selected for examination in terms of gross pathology and histopathology. In addition, on days 0,3,6,9 and 12 following exposure to influenza virus, lungs and spleens were removed and weighed from four mice representing each of the following groups: Flu, Flu/SAD, SAD, UC, Flu/VC, and VC. Following inflation of the lungs with 10% formalin solution, the lungs and spleen specimens were fixed in the formalin solution. The tissues were dehydrated in ethanol, embedded in paraffin, and sectioned into 6 μm sections. Sections were stained with hemotoxylin and eosin and examined by light microscopy. The tissues were scored on a scale of 0-4 depending on the severity of the pathology observed.

The moribund SAD mice were examined for the lung pathology, the hemorrhages and atelectasis, which had been reported by Reddy et al., (1979). Small focal areas of intraalveolar hemorrhage and small areas of atelectasis were present, but were attributed by the pathologist to be artifacts produced at necropsy.

Mice in the Flu/SAD and Flu groups exhibited characteristic signs of viral pneumonia, but no difference were observed between the two groups. Flu/SAD mice also had mutifocal hepatic necrosis and fibrinous peritonitis characteristic of SAD, as did the SAD mice (Fleischhacker et al., in press).

d. Humoral response

1) Antibody production to influenza virus.

The hemagglutination inhibition assay (HIA) was used to determine the titer of antibody to the influenza virus in exposed mice. The stock virus titer was determined by adding 0.25 ml of two-fold virus dilution with 0.25 ml PBS and 0.25 ml of 0.5% human type O red blood cells. The mixture was allowed

to incubate one hour at room temperature. The virus titer was determined as the reciprocal of the highest virus dilution showing positive hemagglutination. The virus dilution used for the hemagglutination inhibition assay was 1/4 of the virus titer.

Mice from Flu, Flu/SAD, SAD and untreated controls groups were bled 21 days after exposure to influenza virus aerosols. The serum from five mice was pooled and heat inactivated at 56°C for 30 minutes. The serum was diluted 1:5 in PBS and 0.5 mL was absorbed with 0.5 mL of 25% kaolin [Flow Laboratories, McLean, VA]. This suspension was incubated at room temperature for two hours with occassional mixing. After centrifugation at 1000 x g for 10 minutes, the supernatant was removed. The serum was then absorbed with 0.1 mL of packed human type O red blood cells for one hour at room temperature. Following centrifugation, the supernatant was removed and two-fold dilutions were made. In V-bottom microtiter plates; 0.05 mL of serum dilution, 0.05 mL of diluted virus, and 0.05 ml of 1% human type O red blood cells were added. The plates were incubated at room temperature for one hour. The hemagglutination titers were read as the reciprocal of the highest dilution of serum causing inhibition of hemagglutination.

The mean serum titer for those mice exposed to the virus only (Flu) was 332, while in those mice receiving SAD and virus (Flu/SAD), the titer was 187. The titer for the Flu/SAD mice was significantly ($p < 0.05$) less than the titer for the Flu mice. Those animals receiving SAD only had a titer of 5.5 and the control animals showed no antibody whatsoever.

2) Ability to produce antibodies (IgM)

Plaque-forming cell assay and sheep red blood cells (SRBC) antibody titrations were performed using animals in the six experimental groups. Spleen cells were assayed using the Cunningham modification of the Jerne

plaque-forming cell (PFC) assay for IgM producing cells (Cunningham and Szenberg, 1968). The mice were immunized by intraperitoneal injection with 0.5 mL of a 10% suspension of sheep red blood cells (SRBC) [Flow Laboratories, McLean, VA] in phosphate-buffered saline (PBS) on either day 0, 3, 6, 9 or 12 following virus exposure. On the assay day (five days after injection of SRBC; corresponding to days 5,8,11,14 or 17 following influenza exposure), the mice were anesthetized with ether, the axillary artery exposed by blunt dissection, severed and blood collected. The serum was separated and stored at 0°C until antibody titrations were determined.

The spleens were removed from the animals, and homogenized individually in PBS to produce a suspension of single cells. The cells were pelleted by centrifugation (1000 x g), resuspended in RPMI 1640 [Flow Laboratories McLean, VA], and counted using trypan blue to determine the number of viable cells. The spleen cell suspension was adjusted to yield a final concentration of 5 X 10^5 viable cells/mL. In a test tube, 150 µL of the spleen cell suspension, 25 µL guinea pig complement [M. A. Bioproducts, Walkersville, MD] was diluted 1:3 in RPMI 1640 and 25 µL of a 10% SRBC suspension also in RPMI 1640 were mixed. This suspension was transferred into a slide chamber consisting of two microscope slides held together by double-faced masking tape [3M Co. Minneapolis, MN] and divided into three areas. The chambers were filled and sealed with 50% paraffin-50% vaseline. Slides were incubated for one hour at 37°C. Plaques, representing zones of SRBC lysis, were counted using a dissecting microscope at 20x magnification. The results of the plaque assay were expressed as the number of PFC/10^6 spleen cells. Plaque assay results using spleen cells from experimental animals were compared to those of the control animals.

Mice given Flu or Flu/DMSO exhibited higher PFC values throughout the course of the experiment when compared to results obtained with mice given SAD or Flu/SAD (figure 2). Secalonic acid D showed a significant decrease in the number of PFC as compared to the DMSO control (VC) on days 11 and 14. For the SAD group, the PFC were decreased by 45% on day 11 and by 39% on day 14. No mice in the Flu/SAD group were available for assay on day 11, but on day 14 the PFC were decreased by 55%. All groups had values equal to the control on day 17 (Fleischhacker, unpublished dissertation).

e. Cell-mediated immune response - Lymphocyte blastogenesis

Mice from the six experimental groups were randomly selected for study on days 0, 3, 6, and 9 following exposure to the influenza virus. Spleens were removed, placed in PBS and homogenized. The cells were centrifuged (1000 x g) in 50 mL-tubes for ten minutes. The pellet was resuspended in RPMI 1640 and the number of viable cells determined by trypan blue exclusion. The suspension was again pelleted and resuspended to yield a concentration of 2.5×10^6 viable cells/mL in RPMI 1640 medium containing 10% heat inactivated fetal bovine serum (FBS) [KC Biological, Inc., Lenexa, KS], 100 units/mL Penicillin G [Sigma Chemical Co., St. Louis, MO], 100 mg/mL Streptomycin sulfate [Sigma Chemical Co.] and 300 mg/L L-glutamine [KC Biological, Inc.] (complete RPMI 1640). Spleen cells (200 µL) were cultured along with 50 µL of either lipopolysaccharide (LPS) [Sigma Chemical Co.], concanavilin A (Con A) [Calbiochem, San Diego, CA] or pokeweed mitogen (PWM) [GIBCO, Grand Island, NY]. The optimal concentration of the mitogens was determined in preliminary studies to be 5 µg/mL for LPS, 4 µg/mL for Con A and 1:50 dilution of PWM. All cells were cultured in triplicate. Optimal responsiveness occurred 3 days after exposure. Therefore, all plates were incubated at 37°C in a 5% CO_2

incubator of 72 hours. After this time, the plates were pulsed by adding 10 µL/well of ^{3}H-thymidine [ICN Pharmaceuticals, Inc., Irvine, CA] at a concentration of 10 mCi/mL and allowed to incubate an additional 16 hours. Cells were harvested on glass fiber filters with an automatic microharvester [Bellco Glass, Inc., Vineland, NJ]. The filters were dried overnight and placed into individual vials of scintillation fluid (0.3 g/l POPOP and 5 g/lk PPO [Sigma Chemical Co.] Samples were counted in a Beckman LS 1800 Liquid Sciintillation counter. Results were expressed as counts per minute (cpm) ± standard error of the mean (S.E.M.), as well as the stimulation index (cpm experimental/cpm control x 100). The Duncan's Multiple Range test determined statistically if any differences existed between the experimental and control groups. Those data sets with a p value less then 0.05 were designated as being significantly different from the control.

The in vitro response of spleen cells from Flu and Flu/DMSO mice to LPS was comparable to the responses of VC and UC on days 3 and 6. However, on day 6 the responses of the Flu/SAD group were suppressed 65%, while those of the SAD group were suppressed only 27% on day 6. By day 12 (7 days after SAD injection), the response of the SAD group to LPS was suppressed 90% (Fleischhacker, unpublished dissertation).

In response to Con A, the Flu group had increased lymphocyte blastogenesis in vitro by three days after virus exposure and was increased through day 12. However, six days after virus exposure the response of the Flu/SAD group was suppressed by 27% and further suppressed by 64% nine days after virus exposure. Suppression of the blastogenesis response in the SAD group paralleled the suppression in the Flu/SAD group, but was less severe, 7% on day six (one day after SAD injection) and 59% on day nine (four days after SAD injection).

When PWM was added to spleen cells, the blastogenesis response of spleen cells from the Flu and Flu/DMSO groups were increased or comparable to the control during the whole 12-day period. Six days after virus exposure (one day after SAD injection), the responses from both Flu/SAD and SAD groups were suppressed by more than 80%. The responses remained suppressed by 75% nine days after virus exposure (four days after SAD injection) (Fleischhacker, unpublished dissertation).

2. Immunotoxic properties of SAD

Animals used in the second phase of the study were male Balb/C mice [Harlan Sprague-Dawley, Inc., Houston, TX] weighing approximately 20 grams. The mice were housed in plastic cages in groups of five for at least one week prior to treatment. The cages were maintained in a temperature-controlled room at 18°C with artificial illumination (12-hour light/dark cycle). Pelleted feed and fresh water were available ad libitum.

a. Humoral immune response

1) Ability to produce antibodies (IgM)

Plaque-forming cell (PFC) assay was performed as previously described using animals administered either 5, 9, 11, 15, 23 or 35 mg/kg of SAD. Mice were injected intraperitoneally with the SRBC suspension on either day 0, 1, 4 or 7 following SAD administration. The assay was performed five days later.

Mice immunized with SRBC for 5 days and administered SAD immediately or 3 days prior to sacrifice exhibited an enhancement of PFC responsiveness (Table 5). Mice given SAD 24 hours prior to SRBC immunization exhibited suppression of antibody reactivity to SRBC. Dosages of 5 to 11 mg/kg of SAD were effective in producing suppression of PFC responsiveness to SRBC.

2) Sheep red blood cell antibody titrations

The serum samples that were collected from the mice immunized with SRBC in the previous section were heat inactivated at 56°C for 30 minutes prior to the hemagglutination assay. The serum was placed into hemagglutination plates and diluted two-fold with PBS yielding a final volume of 100 µL per well. In addition, 100 µL of a 1% SRBC suspension in PBS was added to each well. Plates were incubated for one hour at 37°C. Each well was examined for hemagglutination and scored as a positive or negative response. Antibody titers were expressed as the reciprocal of the highest dilution that yielded positive hemagglutination and as a percent of control.

Mice administered SAD at concentrations of 9, 11, and 15 mg/kg showed a decrease in antibody titer to SRBC until day 6, at which time, enhancement of the antibody response was observed. As in the PFC experiment, the lowest antibody titer seen at all dosages of SAD was on day 6, and was 50% of the titers from control animals (Fleischhacker, unpublished dissertation).

b. Cell-mediated immune response: Lymphocyte blastogenesis

Lymphocyte blastogenesis was performed as previously outlined using cell suspensions prepared from spleen or axillary, cervical, brachical and inguinal lymph nodes from mice administered either 10 or 35 mg/kg of SAD. The assay was performed on days 1, 4, and 7 following administration of SAD. Cells were cultured with either LPS, PWM or phytohemagglutinin-A (PHA) [Difco Laboratories, Detroit, MI].

Analysis of lymph node cell responsiveness to Con A revealed no significant difference in reactivity of cells from control mice and mice administered 10 or 35 mg/kg SAD. Reactivity of cells from SAD treated mice were comparable to controls, except as day 1 and 7 following administration of 35 mg/kg of SAD, at which time, a 30% and 33% suppression of activity was observed.

The PWM reactivity of lymph node cells from animals administered 10 mg/kg of SAD on days 1 and 4 after SAD was 60% of control values. On day 7, the response was comparable to that of the control. At a dose of 35 mg/kg, 36% enhancement was seen at day 1, and 24% suppression was observed on day 7.

Lymph node cells from all mice given SAD, regardless of dosage, exhibited suppression of responsiveness to PHA. This ranged from 71% suppression at 10 mg/kg to 52% at 35 mg/kg on day 1. The PHA responsiveness of cells from mice given 35 mg/kg or 10 mg/kg SAD were comparable to control reactivity. At day 7, 32% suppression of responsiveness was observed at a dose of 35 mg/kg, whereas, enhancement of responsiveness was seen with cells from animals given 10 mg/kg.

Spleen cells from mice administered 10 mg/kg of SAD and incubated with Con A showed a response equal to spleen cells from untreated controls at days 1 and 4. On day 7, a slight enhancement in proliferation of the cells was observed. Values in the range of the control group were observed on day 1 and 4 in the group of mice receiving 35 mg/kg of SAD. On day 7, the group showed a 35% suppression in activity.

On day 1, those spleen cells from animals administered SAD and exposed to PWM had values in the same range as the untreated controls. On day 4, the reactivity of cells from the group given 35 mg/kg was unchanged. However, enhancement of responsiveness to PWM on day 4 was observed with cells from mice given 10 mg/kg SAD. By day 7, suppression of 24% in the 20 mg/kg group and 40% in the 10 mg/kg was observed.

Spleen cell reactivity to PHA on day 1 following SAD administration was decreased 26% in animals given 10 mg/kg SAD and 19% in animals given 35 mg/kg when compared to the control. This was also observed on day 4, but to a lesser

extent (20% suppression). On day 7, a 35% suppression was observed in the 35 mg/kg group. A slight enhancement was observed in the 10 mg/kg group (Fleischhacker, unpublished dissertation).

3. Summary of Immunotoxicity Results

1. Administration of SAD significantly increased the mortality rate of influenza-infected mice, but did not affect the mean time of death.

2. The concentration of viable virus in the Flu/SAD group of mice was higher than the Flu group only on the ninth day after SAD administration.

3. Mice in the Flu/SAD and Flu groups exhibited characteristic signs of viral pneumonia, but no difference was observed between the two groups. Flu/SAD mice also had multifocal hepatic necrosis and fibrinous peritonitis characteristic of SAD. Abnormal SAD induced lung pathology reported in previous studies was not observed in this study.

4. SAD appeared to interfere with the immune response in mice both exposed and unexposed to influenza virus as indicated by the PFC assay, antibody titers to SRBC and influenza virus and lymphocyte blastogenesis.

IV. SUMMARY

These studies were initiated to determine what microorganisms and which of their toxic metabolites were present in grain dust from the terminal grain elevator facilities on the lower Mississippi River. In addition, the occurrence of two pesticides in grain dust was determined. The consequences of exposure to one fungal toxin, secalonic acid D, on host resistance to a viral infection and modulation of the immune functions of laboratory mice were also determined.

The fungi in grain dust from these elevators were similar in number to Duluth-Superior study (Whidden et al., 1980), but the species varied. Few

bacteria were recovered in the respirable dust samples, but up to 53.4 million/g were in settled dust samples (DeLucca et al., 1984). In addition 61% of the Gram-negative bacteria were Enterobacter agglomerans, which produces an endotoxin. Endotoxin concentrations in settled grain dust were high ranging up to 183 ng/mg of dust in one study (DeLucca et al., 1984) and up to 5.6 ng/mg in another study (DeLucca and Palmgren, in review). In both studies, the worst exposure to dust containing the highest concentration of endotoxin would be below the threshhold of endotoxin reported to cause a febrile response or respiratory dysfunction; 2 ng/kg body weight for febrile response (Wolff, 1973) and 10 ng/kg body weight for respiratory dysfunction (Muittari et al., 1980).

Mycotoxins were found commonly in grain dust but at relatively low levels. In the earlier studies, aflatoxin B_1, and ochratoxin were not detected, while zearalenone and secalonic acid D were detected in more than half the samples. In the last study, aflatoxin B_1, was detected in 71% of the samples of settled dust, while zearalenone was detected in only 18%. These results indicated that nearly 90% of all samples contained at least one mycotoxin of the ones we sought and that the occurrence of a particular mycotoxin may vary from year to year as in field occurrences of mycotoxins.

The consequences of exposure to mycotoxins in grain dust were examined by injecting mice with one such toxin, secalonic acid D (SAD), after exposure to an aerosol of influenza virus. The mortality of the joint treatment was significantly greater than the sum of the individual treatments. Therefore, SAD exacerbated the lethal effects of the influenza virus. Futher studies of SAD's effects included mitogen-stimulated lymphocyte blastogenesis and antibody formation in response to sheep red blood cells as measured by the

plaque-forming cell (PFC) assay. The *in vitro* blastogenesis of spleen or lymph node cells from inbred mice exhibited a decreased response to conconavlin A and phytohemagglutinin-P on days 1, 4, and 7 after injection of SAD at 35 mg/kg. Poke weed mitogen caused no significant change from the controls on days 1 and 4, but a decrease in lymphocyte blastogenesis was observed on day 7. The PFC assay revealed an enhanced response 12 to 24 hours after SAD administration with a return to control values by 96 hours.

V. References

Aramanious, M., Cotton, D., Wright, J., Dosman, J., and Carr, I. 1982. Grain dust and alveolar macrophages: An experimental study of the effects of grain dust on the mouse lung. J. Pathol. 136:265-272.

Assoc. Off. Anal. Chem. 1980. Chapter 26. Natural Poisons: Mycotoxins, Official Methods of Analysis of the Assoc. of Off. Anal. Chem., 13th Edition, Assoc. Off. Anal. Chem., Washington, D.C. pp. 414-434.

Blackley, C. H. 1880. Hayfever: its causes, treatment and effective prevention. Experimental Research, London. (original not seen - referred to by Harris, C. H. 1939. Allergy to grain dust and smuts. J. Allergy 10:327-336.)

Burg, W. R., Shotwell, O. L., and Saltzman, B. E. 1981. Measurements of airborne aflatoxins during the handling of contaminated corn. Amer. Ind. Hyg. Assoc. J. 42:1-11.

Chan-Yeung, M., Ashley, M. J., and Grzybowski, S. 1978. Grain dust and the lungs. Can. Med. Assoc. J. 118:1271-1274.

Chan-Yeung, M., Schulzer, M., MacLean, L., Dorken, E., Tan, F., Lam, S., Enarson, D., and Grzybowski, S. 1981. A follow-up study of the grain elevator workers in the port of Vancouver. Arch. Environ. Health 36:75-81.

Chan-Yeung, M., Wong, R., and MacLean, L. 1979. Respiratory abnormalities among grain elevator workers. Chest 75:461.

Christensen, C. M. 1957. Deterioration of stored grains by fungi. Bot. Rev. 23:108-134.

Christensen, C. M., and Kaufmann, H. H. 1965. Deterioration of stored grains by fungi. Ann. Rev. Phytopathol. 3:69-84.

Ciegler, A., Hayes, A. W., and Versonders, R. F. 1980. Production and biological activity of secalonic acid D. Appl. Environ. Microbiol. 39:285-287.

Cinkotai, F. F., Lockwood, M. G., and Rylander, R. 1977. Airborne microorganisms and prevalence of byssinostic symptoms in cotton mills. Am. Ind. Hyg. Assoc. J. 38:554-559.

Cole, R. J., Kirksey, J. W., Dorner, J. W., Wilson, D. M., Johnson, J. C., Johnson, A. N., Bedell, D. M., Springer, J. P., Chexal, K. K., Clardy, J. C., and Cox, R. H. 1977. Mycotoxins produced by Aspergillus fumigatus species isolated from molded silage. J. Agric. Food Chem. 25:826-830.

Cotton, D. J., and Dosman, J. A. 1978a. Grain dust and health. I. Host factors. Ann. Intern. Med. 88:840-841.

Cotton, D. J., and Dosman, J. A. 1978b. Grain dust and health. III. Environmental factors. Ann. Intern. Med. 89:420-421.

Cunningham, A. J., and Szenberg, A. 1968. Further improvements in the plaque technique for detecting single antibody - forming cells. Immunol. 14:599-600.

Darke, C. S., Knowelden, J., Lacey, J., and Ward, A. M. 1976. Respiratory disease of workers harvesting grain. Thorax 31:294-302.

Davies, R. J., Green, M., and Schofield., N. McC. 1976. Recurrent noctornal asthma after exposure to grain dust. Amer. Rev. Respir. Dis. 114:1011-1019.

DeLucca, A. J., II, Godshall, M. A., and Palmgren, M. S. 1984a. Gram-negative bacterial endotoxins in grain elevator dusts. Amer. Ind. Hyg. Assoc. J. 45:336-339.

DeLucca, A. J., II, and Palmgren, M. S. (in review). Seasonal variation in aerobic bacterial populations and endotoxin concentrations in grain dusts Amer. Ind. Hyg. Assoc. J. (in review).

DeLucca, A. J., II, Palmgren, M. S., and Ciegler, A. 1982. Bacillus thuringiensis in grain elevator dusts. Can. J. Microbiol. 28:452-456.

DeLucca, A. J., II, Palmgren, M. S., and de Barjac, H. 1984b. A new serovar of Bacillus thuringiensis from grain dust: Bacillus thuringiensis serovar colmeri (Serovar 21).

Dennis, C. A. R. 1971. Health hazards and grain storage. International Symposium on the Storage of Grain and Its Products. Winnipeg, Manitoba, Canada. June 8-10.

do Pico, G. A. 1979. Grain dust and health. Chest. 75:416.

do Pico, G. A., Jacobs, S., Flaherty, D., and Rankin, J. 1982. Pulmonary reaction to durum wheat, a constituent of grain dust. Chest 81:55-61.

do Pico, G. A., Reddan, W. G., Flaherty, D., Tsiatis, A., Peters, M. E., Rao, P., and Rankin, J. 1977. Respiratory abnormalities among grain handlers. Amer. Rev. Respir. Dis. 115:915-927.

do Pico, G. A., Reddan, W. G., Tsiatis, A., Flaherty, D., Jacobs, S., and Rankin, J. (1981). Pulmonary reaction to grain dust and its constituents. Chest 80 (Supplement): 57S-60S.

Dosman, J., Cotton, D., Graham, B., Li, R., Froh, F., and Barnet, G. 1981. Chronic bronchitis and decreased forced expiratory flow rates in lifetime nonsmoking grain workers. Chest (Supplement) 80:33S.

Dutkiewicz, J. 1978a. Exposure to dust-borne bacteria in agriculture. I. Environmental studies. Arch. Environ. Health Sept/Oct:250-259.

Dutkiewicz, J. 1978b. Exposure to dust-borne bacteria in agriculture. II. Immunological studies. Arch. Environ. Health Sept/Oct:260-270

Dvorackova, I. 1976. Aflatoxin inhalation and alveolar cell carcinoma. Brit. Med. J. 3:691.

Ehrlich, K. C., Lee, L. S., Ciegler, A., and Palmgren, M.S. 1982. Secalonic acid D: Natural contaminant of corn dust. Appl. Environ. Microbiol. 46:99.

Ehrlich, K. C., DeLucca, A. J. II, Ciegler, A. 1984. Naphtho-γ-pyrone production by Aspergillus niger isolated from stored cottonseed. Appl. Environ. Microbiol. 48:1-4.

Emanuel, D. A., Wenzel, F. J., and Lawton, B. R. 1975. Pulmonary mycotoxicosis. Chest 67:293-297.

Eppley, R. M., Stoloff, L., and Campbell, R. D. 1968. Collaborative study of "A versatile procedure for assay of aflatoxins in peanut products," including prepartory separation and confirmation of identify. J. Assoc. Off. Anal. Chem. 51:67-73.

Fairchild, E. J., (ed). 1977. Agricultural Chemicals and Pesticides. A Subfile of the NIOSH Registry of Toxic Effects of Chemical Substances, USDHEW, NIOSH, Cincinnati, OH.

Farant, J.-P. and Moore, C. F. 1978. Dust exposures in the Canadian grain industry. Amer. Ind. Hyg. Assoc. J. 39:177-194.

Farant, J.-P., Moore, C. F., and Windish, J. P. 1973. Airborne fungal spores in Canadian grain elevators. Health and Welfare Canada Report No. HHAW73-2.

Fawcett, R. 1938. Brit. J. Radiol. 11:378.

Fleischhacker, D. S. (unpublished dissertation). Effect on secalonic acid D on influenza-infected mice and its immunotoxic properties. Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

Fleischhacker, D. S., Akers, T. G., Katz, S. P., and Palmgren, M. S. (in press). Enchancement of influenza virus infection by secalonic acid D. Environ. Health Persp. (in press).

Goynes, W. R., Ingber, B. F., and Palmgren, M. S. (in press). Microscopical comparison of cotton, corn, and soybean dusts. Environ. Health Persp. (in press).

Harris, L. H. 1939. Allergy to grain dusts and smuts. J. Allergy 10:327-336.

Heatley, T. F., Kahn, D., and Rex, C. R. 1944. A case of silicosis caused by wheat dust. JAMA 124:980-981.

Kozlowski, T. T. (Ed.). 1972. Seed Biology. Chapter 4. "Insects attacking seeds during storage". Academic Press, Inc. New York. pp247-297.

Krantz. G. W. 1970. A manual of Acarology. Oregon State University Boot Sotres, Corvallis, Oregon. pp. 335.

Lacey, J. 1980. The microflora of grain dusts, in Occupational Pulmonary Disease: Focus on Grain Dust and Health (Eds. J.A. Dosman and D. J. Cotton), pp. 417-440, Academic Press, N. Y.

Lee, L. S., and Catalano, E. A. 1981. Pons scaled-down clean-up column adapted for use in solvent saving modification of the CB method for aflatoxin. J. Am. Oil Chem. Soc. 58:949A-951A.

Mislivec, P. B., and Tuite, J. 1970. Species of Penicillium occurring in freshly-harvested and in stored dent corn kernels. Mycologia 62:67-74.

Morey, P., Fischer, Jr., and Rylander, R. 1983. Gram-negative Bacteria on Cotton with Particular Reference to Climatic Conditions. Am. Ind. Hyg. Assoc. J. 44:100-104.

Moselhi, M., El-Gazzar, R., Abdel-Kader, H. M., El-Sadik, M., and El-Dakhakhny, M. (1979). Clinical and biological investigations among grain handlers. J. Egypt. Pub. Health Assoc. 54:396-414.

Muica, N., and Teculescu, D. 1977. Respiratory disease in grain handlers. Rev. Roum. Med.-Med. Int. 15:173-181.

Muittari, A., Rylander, R., and Salkinoja-Salonen, M. 1980. Endotoxin and bath water fever. Lancet ii:89.

Murray, W. J. 1979. Methods of overcoming merchandising problems associated with excessive pesticide residues in grain dust, in Proceedings of the International Symposium on Grain Dust (Eds. B. S. Miller and Y. Pomeranz), pp.309-320, Division of Continuing Education, Kansas State University, Manhattan, KS.

Olenchock, S. A. and Major, P. C. 1979. Immunological studies of grain dust. J. Environ. Pathol. Toxicol. 2:9-22.

Olenchock, S. A., Mull, J. C., Major, P. C., Gladish, M. E., Peach, M. J., III, Pearson, D. J., Elliot, J. A. and Mentnech, M. S., 1978a. Activation of the alternative pathway of complement by grain. Clin. Allergy 8:12-133.

Olenchock, S. A., Mull, J. C., Major, P. C., Peach, M. J., III, Gladish, M., and Taylor, G. 1978b. In vitro activation of the alternative pathway of complement by settled grain dust. J. Allergy Clin. Immunol. 62:295-300.

Ordman, D. 1958. Cereal grain dust as a cause of respiratory allergy in South Africa. S. Afr. Med. J. 32:784-788.

Palmgren, M. S. (1985). Microbial and Toxic Constituents of Grain Dust and their health implications. In Trichothecenes and Other Mycotoxins (Ed. J. Lacey) pp. 47-56, Wiley and Sons, Ltd., London.

Palmgren, M. S., DeLucca, A. J., II, Klich, M. A., and Ciegler, A. 1983a. Fungi and Bacteria in Grain Dust from New Orleans Area Grain Terminals, Chap. 40 in Aerosols in the Mining and Industrial Work Environment, V. A. Marple and B. Y. H. Liu, eds., pp. 549-557, Ann Arbor Science Publication, Ann Arbor, MI.

Palmgren, M. S., DeLucca, A. J., II, Lee, L. S., and Ciegler, A. 1983b. Preliminary study of mycoflora and mycotoxins in grain dust from New Orleans area grain elevators. Amer. Ind. Hyg. Assoc. J. 44:485-488.

Palmgren, M. S., and Lee, L. S. (in press). Separation of mycotoxin - containing sources in grain dust and determination of their mycotoxin potential. Environ. Health Persp. (in press).

Palmgren, M. S. and Lee, T. C. 1984. Malathion and diazinon levels in grain dust from New Orleans area grain elevators. Amer. Ind. Hyg. Assoc. J. 45:168-171.

Paulsch, H. P., van Egmond, W. E., and Schuller, P. L. 1982. Estimation of ochratoxin A in cereal grains. International IUPAC Symposium on Mycotoxins, Vienna, Austria, September 1-3.

Ramazzini, B. 1713. DeMorbus Artificum Diatriba, (Transl. W. C. Wright), Chicago, IL (1940).

Rankin, J., do Pico, G. A., Reddan, W. G., and Tsiatis, A. 1979. Respiratory disease in grain handlers, in Proceedings of the International Symposium on Grain Dust (Eds. B. S. Miller and Y. Pomeranz), p. 91, Division of Continuing Education, Kansas State University, Manhattan, KS.

Reddy, C. S., Hayes, A. W., Williams, W. L., and Ciegler, A. 1979. Toxicity of secalonic acid D. J. Toxicol. Environ Health 5:1159-1169.

Reddy, C. S., Reddy, R. V., and Hayes, A. W. 1981. High-performance, liquid chromatographic analysis of the mycotoxin secalonic acid D and its application to biological fluids. J. Chromatog. 208:17-26.

Rylander, R., and Morey, P. 1982. Airborne endotoxin in industries processing vegetable fibers. Am. Ind. Hyg. Assoc. J. 43:811-812.

Rylander, R., and Vesterlund, J. D. 1982. Airborne Endotoxins in Various Occupational Environments. In: Endotoxins and Their Detection With the Limulus Amebocyte Lysate Test. S. Watson, J. Levin, and T. Novitsky, eds. pp. 399-409. Alan R. Liss, New York.

Saleh, S. M., Harris, R. T., and Allen, O. N. 1969. Method for determining Bacillus thuringiensis var. thuringiensis Berliner in soil. Can. J. Microbiol. 15:1101-1104.

Samsonov, P. F. 1960. Respiratory mycotoxicoses (pneumomycotoxicoses), in Mycotoxicoses of Man and Agricultural Animals (Ed. V. I. Bilay), pp. 131-140, U. S. Joint Publication Research Service, Office of Technical Services, U. S. Department of Commerce, Washington, D. C.

Samsonsov, P. F. and Samsonov, A. P. 1960. The respiratory mycotoxicoses (pneumomycotoxicoses) experimentally, in Mycotoxicoses in Man and Agricultural Animals, (Ed. V. I. Bilay), pp. 140-151, U. S. Publication Research Service Office of Technical Services, U. S. Department of Commerce, Washington, D. C.

Sheridan, D., Deutscher, C., Tan, L., Taybank, J., Gerrand, J., Horne, S., Yoshida, K., Barnett, G. D., Cotton, D. and Dosman, J. 1977. The relationship between exposure to cereal grain dust and pulmonary function in grain workers.

Skoulas, A., Williams, N., and Merriman, J. E. 1964. Exposure to grain dusts. II. A clinical study of the effects. J. Occup. Med. 6:359-371.

Sorenson, W. G., Green, F. H. Y., Vallyathan, V., and Ciegler, A. 1982. Secalonic acid D Toxicity in rat lung. J. Toxicol. Environ. Health 9:515-525.

U. S. Environmental Protection Agency. 1980. Code of Federal Regulations, 40, CFR 180.153.

U. S. Environmental Protection Agency. 1982. Part 180 - Tolerances and Exemptions from Tolerances for Pesticide Chemicals in or on Raw Agricultural Commodities. Fed. Reg. 47:25960.

Wallace, H. A. H. 1973. Chapter 4: Fungi and other orgainisms associated with stored grain. In Grain Storage: Part of a System. R. N. Sinha and W. E. Muir (Eds.). Avi Publishing Co., Inc. pp. 71-98.

Ware, G. M., and Thorpe, C. W. 1978. Determination of zearalenone in corn by high pressure liquid chromatography and fluorescence detection. J. Assoc. Off. Anal. Chem. 61:1058-1062.

Warren, P., Cherniack, R. M., and Tse, K. S. 1974. Hypersensitivity reactions to grain dust. J. Allergy Clin. Immunol. 53:139-149.

Warren, C. P. W. and Manfreda, J. 1980. Respiratory abnormalities in grain workers 1971-1977: A preliminary analysis, in Occupational Pulmonary Disease: Focus on Grain Dust and Health (Eds. J. A. Dosman and D. J. Cotton), pp. 417-440, Academic Press, N. Y.

Whidden, M. P., Smalley, E. B., Caldwell, R. W., Phillip, J. K., Mai, S. H., and Burkholder, W. E. 1980. Microbial Flora and Fauna of Respirable Grain Dust from Grain Elevators. NIOSH Contract 210-77-0150 Final Report, NIOSH, ALOSH, Morganstown, W. V.

Wittich, F. W. 1940. Lancet 60:418.

Wolff, S. M. 1973. Biological effects of bacterial endotoxins in man, in Bacterial Lipopolysaccharides (Eds. E. H. Kass and S. M. Wolff), pp. 251-256, University of Chicago Press, Chicago, IL.

VI. ACKNOWLEDGEMENTS

Without the help and cooperation of the scientist, statistical staff, and clerical staff, this project would have been impossible. In particular, the following people at SRRC were especially cooperative; Louise Lee, Anthony DeLucca, II, Ken Ehrlich, A. Ciegler, Margie Chmielewski, Ed Bowers, Kim Daigle, Susan Holland, and Becky Skau. In addition to the people at Tulane who were co-authors, Terry Lee, Deborah Fleischhacker, Tom Akers, and Stephan Katz, the following people at Tulane were of great assistance; Peter Gerone, Ken Soike, Mary Barbee, and Gary Baskin at Tulane's Delta Regional Primate Center and A. J. Englande and Sharon Bock at Tulane's Environmental Health Sciences Department who administered the contract. Also, R. J. Cole of the National Peanut Laboratory, Dawson, GA supplied a standard of fumigaclavine C and confirmed it in a culture extract for us.

VII. Appendix 1.

Tables and Figures

Table 1.

Percent Frequency of Fungi Commonly Isolated from Grain Dust

	During Harvest		During Storage					
	Lacey[1]		Farant[2]		Lacey[1]		Whidden[3]	
	V[4]	NV[5]	V	NV	V	NV	V	NV
Penicillium	93	}36[6]	69	}84-100[6]	100	--[7]	79	--
Aspergillus	<10		43		94	--	94	--
Cladosporium	100	100	46	25-95	82	100	81	68
Mucorles	55	<10	20	50-97	87	--	34	30
Yeasts	32	33	8	--	98	--	80	--
Verticillium	100	67	--	--	--	--	--	--
Alternaria	90	99	29	6-80	52	100	20	7
Botrytis	66	67	3	39-100	3	--	--	--
Ustilago	--	55	--	--	--	98	--	86

[1]Lacey, 1980

[2]Farant et al., 1973 - In this study, viable counts represent % frequency of the most frequently isolated species of the genera listed; non-viable counts were not tabulated for the group of elevators. The range of frequencies of the individual elevators are listed.

[3]Whidden et al., 1980.

[4]V = Viable counts; fungi grown and then counted.

[5]NN = Non-viable counts; direct microscopic counts.

[6]Value represents combined count of Penicillium and Aspergillus spores because most are indistinguishable microscopically.

[7]Not reported.

Table 2.

Concentration of Fungal Spores and Dust in the Air of Grain Elevators

	Duluth-Superior[1] (Range)	Canada[2] (Range)
Fungal Spores (#/m^3)		
Viable	ND[3]-9.4 $x10^4$	ND-9.85 $x10^8$
Non-Viable + Viable	ND-3.6 $x10^7$	---
Resp. Dust (mg/m^3)	ND-8.26	ND-76.3
Total Dust (mg/m^3)	0.14-39.1	0.18-781

[1]Whidden et al., 1980.

[2]Farant et al., 1973; Farant and Moore, 1978.

[3]ND = None detected.

Table 3.

Colony-forming Units in Grain Dust

		Range ($x10^3$/g of Dust)	Mean (±SEM) ($x10^3$/g of Dust)
Elev. I (n=5)	Aspergillus	5- 675	271(±147)
	Penicillium	1- 150	247(± 90)
	Fusarium ND[A]- 20	8(± 4)	
	TOTAL 18-2175	865(±404)	
Elev.II (n=7)	Aspergillus	95-3000	899(±395)
	Penicillium	ND-2200	726(±287)
	Fusarium ND- 125	30(± 17)	
	TOTAL 825-3700	1920(±526)	
Duluth[B] (n=12)	Aspergillus	60-1600	544(±159)
	Penicillium	50-2700	672(±236)
	Fusarium ---[C]	-	
	TOTAL 920-43 800	9893(±7004)	

[A]None detected.

[B]Duluth-Superior grain elevators data; two samples from each of six elevator (Whidden et al., 1980).

[C]Data unavailable.

Table 4.

Spore Counts and Toxin Concentrations.

Fungus	Spores/g	Toxin	Toxin Concentration ng/g of Spores	Toxin Concentration ng/10^6 spores
A. parasiticus	$2.2X10^{10}$	aflatoxin B_1	16600	0.976
A. parasiticus Mutant Nor-1	$1.22X10^{10}$	norsolorinic acid	280	0.023
A. niger	$4.0X10^{10}$	aurasperone C	460,000	0.114
A. fumigatus	$9.4X10^{10}$	fumigaclavine C	930,000	9.89
P. oxalicum	$7.5X10^{10}$	secalonic acid D	1890	0.025

Table 5.

The PFC Response of Splenic Lymphochytes from Balb/C Mice Administered SAD to SRBC[a]

Dose SAD mg/kg	Assay day after injection of SAD			
	0	3	6	9
5	106 ± 9[b]	141 ± 12	55 ± 5	128 ± 7
9	116 ± 14	103 ± 11	39 ± 4	115 ± 6
11	202 ± 16	123 ± 9	44 ± 4	194 ± 10
15	---	116 ± 14	93 ± 5	114 ± 7
23	117 ± 8	139 ± 5	67 ± 4	69 ± 6
35	200 ± 20	75 ± 8	100 ± 12	226 ± 19

a. Mice were immunized with Sheep red blood cells five days prior to assaying.

b. Values are expressed as a percent of control (Plaque Forming Cells (PFC) experimental/PFC control x 100 + S. E. M.)

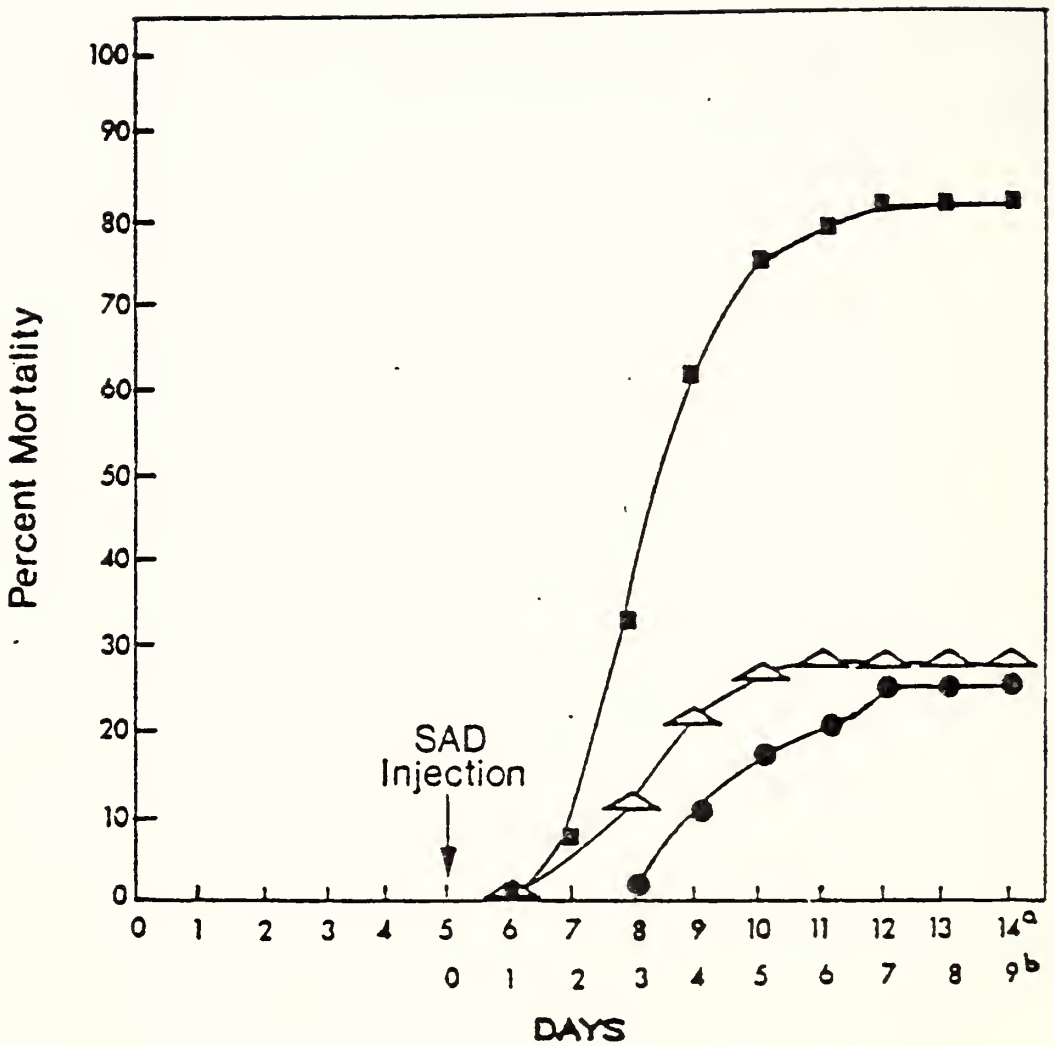

Figure 1. Cumulative mortality of mice administered influenza virus and/or SAD; (●) SAD, (△) Flu, (■) Flu/SAD.
a. Days after influenza virus exposure
b. Days after SAD injection

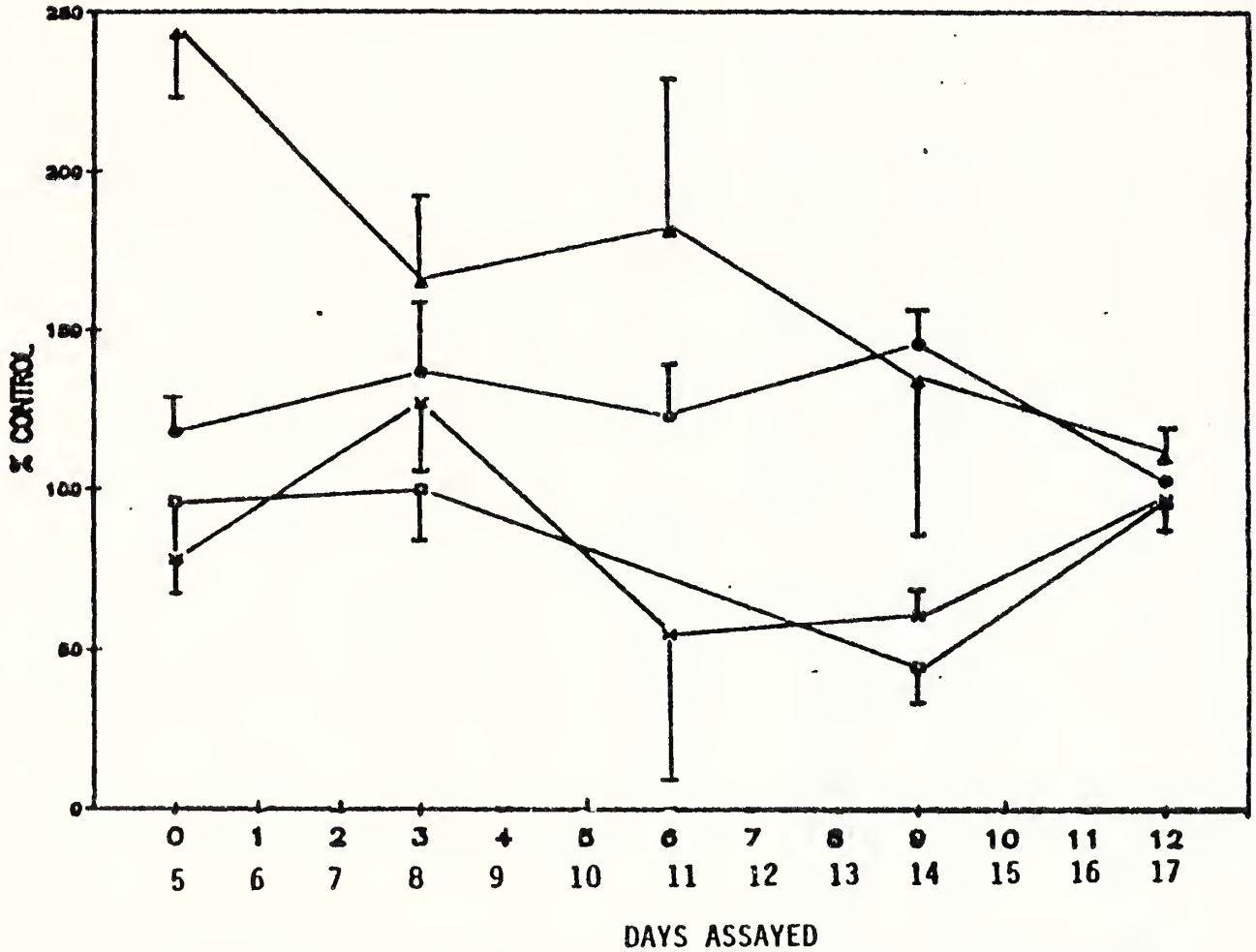

Figure 2. The PFC response of splenic lymphocytes from Balb/C mice administered SAD and/or influenza virus to SAD (mice immunized with SRBC five days prior to assay); (X) SAD, (o) Flu, (▲) Flu/DMSO, (■) Flu/SAD.

VIII. Appendix 2.

List of Publications, Abstracts, and Presentations Resulting from Cooperative Agreement No. 58-7B30-0-216.

I. Publications

Palmgren, M. S., Akers, T. G., Gerone, P. J., and Ciegler, A. 1982. Interaction between Secalonic Acid D and Influenza Virus in Mice. Fed. Proc. 41:1643.

DeLucca, II, A. J., Palmgren, M. S., and Ciegler, A. 1982. Bacillus thuringiensis in Grain Elevator Dusts. Can. J. Microbiol. 28:452-456.

Ehrlich, K. C., Lee L. S., Ciegler, A., and Palmgren, M. S. 1982. Secalonic Acid D: A Natural Contaminant of Corn Dust. Appl. Environ. Microbiol. 44:1007-1008.

Palmgren, M. S., DeLucca, II, A. J., Klich, M. A., and Ciegler, A. 1983. Chapter 40. Fungi and Bacteria in Grain Dust from New Orleans Area Grain Terminals. In Aerosols in the Mining and Industrial Work Environment, Vol. 2: Characterization, ed. by V. A. Marpel and B. Y. H. Liu, Ann Arbor Science Publ., Ann Arbor, MI, PP. 549-557.

Palmgren, M. S. and Ciegler, A. 1983. Chapter 8. Aflatoxins. In Handbook of Natural Toxins, Vol. I: Plant and Fungal Toxins, ed. by A. T. Tu and R. F. Keeler, Marcel Dekker, Inc., pp. 299-323.

Palmgren, M. S. and Ciegler, A. 1983. Chapter 9. Toxicity and Carcinogenicity of Fungal Lactones: Patulin and Pencillic Acid. In Handbook of Natural Toxins, Vol. I: Plant and Fungal Toxins, ed. by A. T. Tu and R. F. Deeler, Marcel Dekker, Inc., pp. 325-341.

Palmgren, M. S., Lee, L. S., DeLucca, II, A. J., and Ciegler, A. 1983. Preliminary Study of Mycotoxins and Mycoflora in Grain Dust from New Orleans Area Grain Elevators. Amer. Ind. Hyg. Assoc. J. 44:485-488.

Palmgren, M. S. and Lee, T. c. 1984. Malathion and Diazinon Levels in Grain Dust from New Orleans Area Grain Elevators. Amer. Ind. Assoc. J. 45:168-171.

DeLucca, II, A. J., Godshall, M. A., and Palmgren, M. S. 1984. Gram-Negative Bacterial Endotoxins in Grain Elevator Dusts. Amer. Ind. Hyg. Assoc. J. 45:336-339.

DeLucca, II, A. J., Palmgren, M. S., and de Barjac, H. 1984. A New Serovar of Bacillus thuringiensis from Grain Dust: Bacillus thuringiensis serovar colmeri (serovar 21). J. Inver. Pathol. 43:437-438.

Ehrlich, K. C., and Lee, L. S. 1984. Mycotoxins in Grain Dust: Method for Analysis of Aflatoxins, Ochratoxin A, Zearalenone, Vomitoxin, and Secalonic Acid D. J. Assoc. Off. Anal. Chem. 67:963-967.

Palmgren, M. S. 1985. Chapter 5. Microbial and Toxic Constituents of Grain Dust and their Health Implications. In Trichothecenes and Other Mycotoxins, ed. by J. Lacey, John Wiley and Sons, Inc., London, pp. 47-57.

Palmgren, M. S., and Lee, L. S. 1985. Separation of Mycotoxin-Containing Sources in Grain Dust and Determination of their Mycotoxin Potential. Environ. Health Persp. (in press).

Fleischhacker, D. S., Akers, T. G., Katz, S. P., and Palmgren, M. S. 1985. Enhancement of Virus Infection by Secalonic Acid D. Environ. Health Persp. (in press).

Goynes, W. R., Ingber, B. F., and Palmgren, M. S. 1985. Microscopical Comparison of Cotton, Corn, and Soybean Dusts. Environ. Health Persp. (in press).

Fleischhacker, D. S. 1985. Effect of Secalonic Acid D on Influenza - Infected Mice and Its Immunotoxic Properties. (Unpublished Dissertation) Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, 111 pages.

DeLucca, A. J., II, and Palmgren, M. S. 1986. Seasonal Variation in Aerobic Bacterial Populations and Endotoxin Concentrations in Grain Dusts. Amer. Ind. Hyg. Assoc. J. (in review).

Whidden, M. P.*, Lee, L. S., and Ciegler, A. 1981. Mycotoxins Mycoflora and Minerals in Grain Dust. 1981. American Industrial Hygiene Conference, Portland, OR, May, 1981.

Ehrlich, K. Palmgren, M., Lee, L. S., and Ciegler, A. 1981. Secalonic Acid D, a Mycotoxin, Is a Natural Contaminant of Corn Dust Obtained from Grain Elevators. South Central Branch of American Society for Microbiology, New Orleans, LA, October, 1981.

DeLucca, A. J., II, Palmgren, M. S., and Ciegler, A. 1981. Bacillus thuringiensis in Grain Dusts. South Central Branch of American Society for Microbiology, New Orleans, LA, October, 1981.

Palmgren, M. S., DeLucca, A. J., II, Klich, M. A., and Ciegler, A. 1981. Fungi and Bacteria in Grain Dust from New Orleans Area Grain Terminals. International Symposium on Aerosols in the Mining and Industrial Work Environment, Minneapolis, MN, November, 1981.

DeLucca, A. J., II, Palmgren, M. S., and Ciegler, A. 1982. Serotypes of Bacillus thuringiensis from Grain Stored in Elevators. American Society for Microbiology Annual Meeting, Atlanta, GA, March, 1982.

Palmgren, M. S., Akers, T. G., Gerone, P. J., and Ciegler, A. 1982. Interaction between Secalonic Acid D and Influenza Virus in Mice. Fed. Proc. 41:1643.

Goynes, W. R., Bogatz, J. A., Lee, L. S., and Palmgren, M. S. 1983. Microscopic Examination of Aspergillus flavus Invasion of Cottonseed. American Society for Microbiology Annual Meeting, New Orleans, LA, March, 1983.

Palmgren, M. S. and Lee, T. C. 1983. Malathion and Diazinon Levels in Grain Dust from New Orleans Area Grain Elevators. Southwestern Association of Toxicologists, New Orleans, LA, May 13-14, 1983.

Palmgren, M. S. 1983. Microbial and Toxic Constituents of Grain Dust and their Health Implications. (Invited Presentation) International Mycotoxin Symposium, Sydney, Australia, August 12-15, 1983.

Palmgren, M. S. 1983. Mycoflora and Mycotoxins in Grain Dust; Immunomodulation by Mycotoxins. Seminar for Department of Microbiology and Immunology, Tulane School of Medicine, New Orleans, LA, October 5, 1983.

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* As of June, 1981, Whidden, M. P. is Palmgren, M. S.

Palmgren, M. S., DeLucca, II, A. J., Fleischhacker, D., and Lee, L. S. 1983. Mycotoxins in Grain Dust. Gulf Coast Section of Society of Toxicolog, Houston, TX, October 21-22, 1983.

Palmgren, M. S. 1984. Toxic Components in Grain Dust. Seminar for Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS, April 2, 1984.

Fleischhacker, D. S., Akers, T. G., Katz, S. P., and Palmgren, M. S. 1984. Combined Effects of Secalonic Acid D and Influenza Virus on Mice. Gulf Coast Section of Society of Toxicology Regional Meeting, New Orleans, LA, June 7-8, 1984.

Palmgren, M. S. and Fleischhacker, D. S. 1984. Occurrence of Mycotoxins in Grain Dust. New Orleans Conference on Agricultural Dusts: I. Cotton and Grain Dusts, New Orleans, LA, November 1 and 2, 1984.

Lee, L. S. and Palmgren, M. S. 1984. Separation of Mycotoxin-Containing Sources in Grain Dust. New Orleans Conference on Agricultural Dusts: I. Cotton and Grain Dusts, New Orleans, LA, November 1 and 2, 1984.

Goynes, W. R., Ingber, B. F., and Palmgren, M. S. 1984. Microscopic Comparison of Cotton, Soybean, and Corn Dusts. New Orleans Conference on Agricultural Dusts: I. Cotton and Grain Dusts, New Orleans, LA, November 1 and 2, 1984.

DeLucca, A. J., II and Palmgren, M. S. 1984. Seasonal Variations in Bacterial Populations and Endotoxin Concentrations in Dusts from Grain Terminals. New Orleans Conference on Agricultural Dusts: I. Cotton and Grain Dusts, New Orleans, LA, November 1 and 2, 1984.

Fleischhacker, D. S., Akers, T. G., Katz, S. P., and Palmgren, M. S. 1984. Combined Effects of Secalonic Acid D and Influenza Virus on Immune Functions of Mice. New Orleans Conference on Agricultural Dusts: I. Cotton and Grain Dusts, New Orleans, LA, November 1 and 2, 1984.

DeLucca, A. J., II, and Palmgren, M. S. 1985. Seasonal Variations in the Aerobic Microorganisms and Endotoxin Concentrations in Grain Dusts. American Society for Microbiology Annual Meeting, Las Vegas, NV, March 10-14, 1985.

Palmgren, M. S., and Fleischhacker, D. S. 1985. Multiple Mycotoxin Contamination of Grain Dust. Association of Offical Analytical Chemists Spring Workshop, Dallas, TX, April 8-11, 1984. (Invited Presentation)